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THE CARDIAC LESIONS IN LIBMAN-SACKS DISEASE *

WITH A CONSIDERATION OF ITS RELATIONSHIP TO ACUTE DIFFUSE LUPUS ERYTHEMATOSUS

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In 1924 Libman and Sacks [1] described a clinical-pathological syndrome based on 4 cases which bore certain resemblances to both rheumatic fever and subacute bacterial endocarditis. Impressed by an unusual non-rheumatic and non-bacterial valvular and mural endocarditis found in the hearts in these cases, they provisionally termed the disease "atypical verrucous endocarditis." While they clearly depicted a distinct clinical syndrome and even suggested that the endocarditis might not be an essential feature of the disease, the emphasis of their title on the endocarditic process may have tended to minimize the importance of the clinical picture. This probably accounts for the failure to recognize many cases of the disease.

In 2 of the 4 cases which they reported Libman and Sacks noted characteristic cutaneous lesions resembling so-called acute lupus erythematosus. In 1931 Baehr [2] reported 17 cases of non-rheumatic verrucous endocarditis (including the original 4 of Libman and Sacks) in which there were striking vascular lesions in the kidneys and other organs.† In 10 of these cases there were ob-

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† Marked endothelial proliferation of the intima of many of the splenic arterioles with severe narrowing of the lumens was noted by Libman and Sacks in 1 of their cases. In another of their cases, subsequently studied by Baehr, Klemperer

served the butterfly shaped facial lesions of lupus erythematosus, and in 12 cases an extensive erythema on the upper part of the chest anteriorly, and on the elbows, knees and extensor surfaces of the legs and arms. In 2 additional cases a similar clinical picture and similar vascular lesions were noted, but the valvular endocardium appeared to be uninvolved. In 1935 Baehr, Klemperer and Schifrin [3] described 23 cases of acute disseminated lupus erythematosus which came to autopsy. They noted vascular lesions in the kidneys in 18 cases, in 5 of which the vessels of numerous other organs were involved. A non-rheumatic verrucous endocarditis was observed in 13 of the cases, in 5 of which the gross appearance of the lesions appeared to be identical with those described by Libman and Sacks.

Thus, a study of these reports reveals that in the cases of so-called "atypical verrucous endocarditis" of Libman and Sacks there are instances of cutaneous lesions very similar to those of acute lupus erythematosus, and conversely, in the cases of acute lupus erythematosus described by Baehr, Klemperer and Schifrin, there are instances of endocardial lesions identical with those of "atypical verrucous endocarditis." This naturally brings up the question of the relationship between the Libman-Sacks cases and the disease known as acute lupus erythematosus; and more specifically, the relationship and relative significance of the cardiac lesions, the cutaneous lesions and the vascular lesions which have been described.

A comparison of the clinical features of the cases of Libman and Sacks with the clinical features of acute lupus erythematosus as described by Kaposi [4] and numerous subsequent writers reveals striking resemblances aside from the presence of the characteristic "butterfly" skin lesions in both groups of cases. The cases described by Libman and Sacks presented the picture of a general infection characterized by an acute febrile course, constitutional manifestations and a fatal outcome. Similarly, there are fatal cases of lupus erythematosus with disseminated skin lesions, fever, constitutional disturbances and acute course, a group first de-

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and Schifrin, the latter authors observed marked vascular lesions in the kidneys. These kidneys were not available for study at the time that Libman and Sacks published their report. For this reason they have no description of the microscopic appearance of these organs in that case (Case 4).

scribed by Kaposi and segregated by him from those with a chronic and benign course and local skin lesions. A striking feature in the Libman-Sacks cases is the presence of renal symptoms sometimes clinically identical with acute glomerulonephritis with azotemia. Similarly, in the acute cases of lupus erythematosus, the occurrence of glomerulonephritis was reported by Goeckerman,[5] Roxburgh,[6] Madden,[7] Keefer and Felty,[8] and others. Libman and Sacks noted a tendency to purpura in 1 of their cases as indicated by hemorrhages into the inflamed laryngeal mucosa, low platelet counts and marked diminution of clot retractability. In another case there were large red blotches of subcutaneous hemorrhage in the lower extremities and a generalized purpuric rash unlike any they had ever seen in rheumatic fever. In acute lupus erythematosus the occurrence of purpura was observed by Kaposi in 2 of his original cases, and the occurrence of a hemorrhagic tendency with bleeding, purpura and thrombocytopenia has been stressed by a number of recent observers. Both in the cases described by Libman and Sacks and in those of acute lupus erythematosus, the characteristic presence of leukopenia, despite the clinical picture of a general infection, is noteworthy. Still another remarkable resemblance in both these groups of cases is a tendency to inflammation of serous membranes. Thus, clinically, there was polyarthritis in all 4 of the Libman-Sacks cases. In 3 of them a pericardial rub was heard; in all 4 there were signs of pleurisy and effusion (proved in 3); and in 1 of the cases there was ascites. The similar prominence of serous membrane involvement in acute lupus erythematosus was reported by Mook, Weiss and Bromberg,[9] Roxburgh,[6] and Baehr and his co-workers.[3] Finally, a number of other features, including the occurrence of meningismus, convulsions and transient paralyses, and termination by bronchopneumonia or a peculiar coma, are common to both the cases of Libman and Sacks and those of acute lupus erythematosus. It is thus a remarkable fact that although only 4 cases were originally described by Libman and Sacks, these authors observed practically all of the characteristic symptoms of acute lupus erythematosus in their cases, whether the cutaneous lesions were present or not.

Here, then, are two groups of cases with striking clinical resemblances, in one of which the "atypical verrucous endocarditis" and in the other distinctive cutaneous lesions appear to be the

central characteristic feature. A close relationship or an identity of the two is further suggested by the presence of skin lesions closely resembling those of acute lupus erythematosus in 2 of the Libman-Sacks cases, and by the presence of the characteristic endocardial lesions of the Libman-Sacks syndrome in several cases of acute lupus erythematosus. In addition, similar striking vascular lesions were found in both groups of cases. However, a common denominator for both groups of cases appears lacking, because neither the characteristic skin lesions of lupus erythematosus, the peculiar gross endocardial lesions of Libman and Sacks, nor the vascular lesions described by Baehr and his co-workers were present in all cases.

In 1932 [10] I described the valvular, endocardial and myocardial lesions in 11 cases of "atypical verrucous endocarditis," 7 of which had skin lesions like those in acute lupus erythematosus. These cases were chosen on the basis of clinical and pathological features corresponding to those described by Libman and Sacks, whose original 4 cases were included. It was shown in this report that the Libman-Sacks cases possessed in an extraordinarily widespread form certain characteristic and even specific microscopic lesions in the heart. It seemed of interest, therefore, to examine the hearts from the cases of lupus erythematosus reported by Baehr, Klemperer and Schifrin in order to determine the nature of the cardiac lesions present in them and, if possible, to throw further light on the relationship of these two syndromes.

Material and Methods

The present report is based on a detailed study of 27 hearts. Twenty-three of these were from fatal cases of acute lupus erythematosus, whether or not the gross endocardial lesions of Libman and Sacks or the microscopic vascular lesions of Baehr and his co-workers were present. Eighteen of the 23 hearts were from the series of cases of diffuse lupus erythematosus reported by Baehr, Klemperer and Schifrin. Five were from more recent cases of the same disease. Four hearts were from cases possessing the clinical and endocardial (atypical verrucous endocarditis) features described by Libman and Sacks in which, however, there was no evidence of lupus erythematosus.

The methods employed in this study have been previously de-

scribed.[10] By cutting blocks according to the standardized method of Gross, Antopol and Sacks,[11] I was able to compare my findings with those in numerous rheumatic and non-rheumatic hearts which have served as the basis of previous communications from this laboratory. The non-rheumatic hearts were from patients who had died of bronchopneumonia and lobar pneumonia, meningococcic meningitis, miliary tuberculosis, bacteremias, luetic aortitis, subacute bacterial endocarditis, uremia, leukemia, periarteritis nodosa, coronary thrombosis and postoperative shock.

Lesions of the Heart in Twenty-three Cases of Diffuse Lupus Erythematosus

In at least 5 of the cases the interpretation of the cardiac lesions was complicated by the existence of strong evidence for an old rheumatic infection. This evidence included a history of rheumatic fever, clinical and pathological signs of mitral stenosis and certain microscopic lesions described elsewhere. In 1 additional case there was a history of rheumatic heart disease without distinct clinical or pathological verification, and in 2 others certain of the lesions were possibly due to an unrecognized attack of rheumatic fever. In still another case there were typical vascular lesions of periarteritis nodosa, which may or may not have been independent of the disease under consideration. Finally, there were 5 cases (1 of which was mentioned above as definitely and 1 as probably rheumatic) in which a terminal or preterminal complicating bacteremia may have modified the pathological picture. In only 1 of these were bacterial vegetations found on one valve. The significance of rheumatic fever as a complicating factor in at least 5 and possibly 7 cases will be taken up in the discussion.

Auricular Lesions

In only 2 cases, characteristic elevated "plateau" lesions, indicative of a healed rheumatic infection, were found macroscopically on the posterior wall of the left auricle. Microscopically, endocardial "reduplications" (Gross [12]) were observed in 9 of the 23 cases. Five of the 9 were cases considered to have been definitely rheumatic, and 2 others were possibly rheumatic. In the remaining 2 cases there were none of the rheumatic stigmata aside from

the auricular reduplications, but there had been a preterminal bacteremia. The reduplications generally consisted of a slender, newly-formed layer at the surface, composed of an edematous, myxomatous or fibrogelatinous tissue which was usually rich in histiocytes. While some of the features often observed in rheumatic reduplications were absent in these cases, these reduplications could not be distinguished from those of rheumatic etiology. Most of them actually occurred in cases considered rheumatic on other pathological and clinical grounds, but in at least 2 of the cases it was possible that the disease under consideration produced auricular reduplications.

Definitely non-rheumatic verrucous endocardial lesions were observed grossly in the right auricle twice, and in the left auricle once. These appeared as flat, granular, tawny or pinkish, pea-sized masses densely adherent to the underlying endocardium and generally situated between the muscular ridges. In these and in 2 other cases in which there were no gross auricular endocardial verrucae, there were characteristic microscopic lesions on the endocardial surface. These lesions were similar to or identical with the surface lesions to be described as occurring also on the ventricular and valvular endocardium and in the valve pockets. Unlike the reduplications, they appear to be rather characteristic of the cases under consideration.

The deeper layers of the auricular endocardium were generally normal except for an occasional increase in histiocytes. In several cases, however, the subendocardium revealed an infiltration with plasma cells and various peculiar mononuclear cells identical with those commonly found throughout the heart in this disease. In 2 cases these cells were especially numerous around areas of fat necrosis. Various characteristic myocardial and pericardial lesions observed in sections of the left auricle will be described below, as they did not differ from the myocardial or pericardial lesions observed elsewhere in the heart and were usually less marked and less frequent than in other sites.

Lesions of the Valve Rings

The valve ring [13] consists of a limited area of fibroelastic tissue at the proximal end of the valve cusp linking it to adjacent cardiac structures. Normally the valve rings contain no polymorpho-

nuclear leukocytes or lymphocytes, and only rarely capillaries. In a previous study we have shown that this is a strategic site from which the rheumatic infection extends into the various valve cusps.[14] In rheumatic fever all of the valve rings generally show inflammatory lesions with polymorphonuclear leukocytes, lymphocytes, mononuclear cells and blood vessels which are frequently of the muscular variety. The mitral, aortic, tricuspid and pulmonic valve rings are involved in that order of frequency, the first three showing lesions almost invariably, whether or not gross vegetations are present.

In the 23 cases under discussion, lesions were observed in the mitral ring 11 times, in the tricuspid ring 12 times, in the pulmonic ring 8 times, and 4 times in the aortic ring. The distribution of ring lesions is even more significant if the definitely rheumatic cases are excluded from the statistics. The mitral, pulmonic and tricuspid rings are then each involved 6 times, and the aortic ring only once. Thus, in these cases, in contrast with rheumatic fever, the pulmonic and tricuspid rings are involved with practically the same frequency as the mitral, while the aortic ring is less frequently affected. Another distinction from the rheumatic process is seen in the relationship between the occurrence of ring lesions, diffuse valvulitis and gross vegetations. In rheumatic fever a ring lesion is always present when valvulitis and verrucae occur in a given valve, and sometimes ring lesions are present when the latter are absent. In the cases herein described, ring lesions were present in only about one-third of the cases in which the remainder of the valve was involved, and in these cases they seemed to occur by extension from widespread pericardial or valvular lesions.

The ring lesions also presented qualitative differences from those observed in rheumatic fever. In most instances they were extremely mild, often consisting only of a scattering of plasma and large mononuclear cells, occasionally with a few thick walled capillaries (Fig. 1). In the active ring lesions the blood vessels also consisted essentially of capillaries of the granulation tissue endothelial bud type (Fig. 2). The endothelial cells showed marked swelling and proliferation, causing the walls to appear thickened and the lumen markedly narrowed or obliterated. Vessels with muscular walls were rarely observed except in the cases

considered to have been the seat of a preceding rheumatic infection. Lymphocytes, and to a lesser extent, polymorphonuclear leukocytes, which are characteristic of the rheumatic inflammatory exudate, were generally absent or extremely sparse. The most distinctive cells in the ring lesions, as indeed elsewhere in the heart, were the plasma and large mononuclear cells (histiocytes ?). Sometimes plasma cells were present almost exclusively but more often they were exceeded numerically by macrophages, young fibroblasts and atypical large mononuclear cells. The latter contained large, deeply staining nuclei of varied shape and abundant bluish pink cytoplasm (hematoxylin and eosin stain). Sometimes the cytoplasm appeared to have undergone granular degeneration and at other times the nucleus was surrounded by a clear space without visible cytoplasm. Occasionally there were multinucleated cells which seemed to be formed by coalescence of these large mononuclears or plasma cells. Because of the abundant, deeply staining nuclei, the entire ring lesion presented a distinctive bluish appearance. Occasionally the rings appeared quite spongy as a result of being honeycombed by numerous, thin walled, bloodless capillary channels.

Lesions of the Valve Leaflets

Gross Features: The gross appearance of the valvular lesions in this group of cases (Figs. 3 and 4) was identical with that described by me [10] as occurring in "atypical verrucous endocarditis." The verrucae occur chiefly in three forms termed respectively the "pyramidal ridge type" resembling the verrucae of rheumatic fever, the "massive thrombotic type" which tends to occur at the commissures and often resembles the vegetations seen in nonbacterial thrombotic and subacute bacterial endocarditis, and the "flat spreading type" which is the most characteristic, if not the most frequent lesion. The latter appears as an extremely flat, granular tawny projection of the endocardium, occurring most frequently at the rings and commissures but usually spreading over both valve surfaces, the valve pockets and even onto the ventricular or auricular endocardium (Fig. 4). Lesions were found grossly on the mitral valve in 10 cases, on the tricuspid in 6, on the pulmonic in 5, and on the aortic in 4.

The location and distribution of the gross lesions may be more

helpful in distinguishing these cases than the appearance of the lesions themselves. Rheumatic verrucae [15] rarely occur on the ventricular mural endocardium and are less frequent and less conspicuous on the pulmonic than on the remaining valves. When present on the pulmonic valve they generally occur on all of the other valves as well, and the latter show well marked valvular deformities. In the cases under discussion gross pulmonic valve verrucae may occur without similar verrucae on the other valves, particularly the aortic, and without gross organic change on those valves. Similarly, the tricuspid valve in rheumatic fever rarely presents verrucae independent of similar verrucae on the aortic or mitral valve. In this disease, however, tricuspid valve verrucae are often present alone, or combined with mitral and pulmonic valve verrucae, while the aortic valve may be grossly normal. Thus, the occurrence of verrucae confined to the valves on the right side of the heart, especially when associated with gross mural lesions, is highly suggestive of the cardiac lesions in acute lupus erythematosus.

Very characteristic also is the location of the verrucae on the valve itself. In rheumatic fever they are generally situated on the closure line of the proximal valve layers (auricular surface of the auriculoventricular valves and the ventricular surface of the semilunar valves). Verrucae on the distal valve layers are uncommon. While pocket verrucae are not uncommon in rheumatic fever, they are either very inconspicuous or observed only on microscopic examination. In acute lupus erythematosus (or "atypical verrucous endocarditis") gross verrucae frequently occur and are generally widespread on the distal valve layers [15] as well as in the valve pockets. The valves must, therefore, be elevated so that these sites may be carefully examined. Furthermore, the verrucae are also frequently present in the pockets of the chordae tendineae attachments, along the chordae themselves and on the chordae attachments to the papillary muscles. The distribution of verrucae in this disease may be similar, in some cases, to that occasionally present in bacterial endocarditis, but the absence of bacteria in the former and microscopic study distinguish the two. Gross ulceration or perforation of valves has not been observed in acute lupus erythematosus or in "atypical verrucous endocarditis." When there are present valvular deformi-

ties apart from those mentioned, a careful search should be made for a previous rheumatic infection.

Microscopic Features: The earliest lesions occur on the superficial layers of the valve leaflets. The ventricularis, auricularis or arterialis layers are equally likely to be involved. In contrast with rheumatic fever, in which the valvulitis precedes the surface alterations, the latter may be present when the deeper layers show little or no abnormalities (Fig. 5). Furthermore, the surface lesions may be continuous over a wide area or scattered over widely separated isolated sites, with some predilection for the region near the pocket of the cusp or near the tip. In rheumatic fever the superficial lesions are markedly localized to the closure line of the leaflets.

The early valvular lesions in acute lupus erythematosus (and in atypical verrucous endocarditis) consist of cellular proliferation and degenerative changes at the surface (Figs. 5, 6, 7, 8 and 13). The flat endothelial cells become cuboidal or rounded and swollen (Fig. 6). The proliferating cells sometimes appear to be endothelial cells but more often they are fibroblasts or large mononuclear cells. The proliferating cells may produce a palisade appearance when they arrange themselves at right angles to the surface of the cusp. Occasionally, this early lesion has a loose spongy appearance due in part to the presence of numerous bloodless vascular channels and in part to the fact that many of the proliferating cells undergo necrosis and appear as bizarre, irregularly shaped, deeply staining nuclei surrounded by clear spaces. Not infrequently there are rows of necrotic mononuclear cells staining a deep blue (hematoxylin and eosin) scattered along the surface. These necrobiotic cells probably represent an early stage of the hematoxylin-stained bodies described by me as occurring in "atypical verrucous endocarditis."[10] Occasionally, the proliferating cells become desquamated from the underlying cell structure. They may then be seen as strips of cells partially separated from the valves, or as polypoid structures still attached to the valve (Figs. 7 and 8).

Quite frequently there are present rather characteristic circular or oval structures apparently separated from the valve but probably representing polyps whose narrow stalks are not included in the section. They may be found on either surface of the cusps

(Figs. 7 and 8) and in the valve or chordae pockets. I have termed these structures eosinophilic multinucleated coalescent bodies. They appear to consist of an eosinophilic hyaline or granular matrix containing deeply staining nuclei oriented in various planes. Certain variations in appearance are sometimes noted. Thus, occasionally the nuclei are found in concentric rings at the periphery of the structure. Sometimes the matrix discloses its origin from plasma cells, young fibroblasts or endothelial cells. In such cases the bodies appear to resemble the proliferating cellular structures found at the surface of the valve itself and thus seem to arise by a coalescence of these cells and from their desquamation en masse. Although these structures bear a close resemblance to, or may be identical with, similar polypoid formations occasionally seen in rheumatic fever and in subacute bacterial endocarditis, they appear to have some special significance in this disease because they are found with unusual frequency not only on one or more of the valves, but also near many other endothelial surfaces within the heart, including the ventricular endocardium (Fig. 9), the aortic and pulmonic intima (Fig. 10), the pericardium (Fig. 11), and within various myocardial and pericardial arteries and veins (Figs. 12 and 17). Furthermore, they are occasionally present in association with characteristic pericardial or myocardial lesions when other more striking valvular lesions are absent. Similar bodies, occurring more discretely, were found in 4 of the 100 non-rheumatic control cases. This suggests that these multinucleated bodies represent the result of an irritation process. Two of the control cases in which these bodies were found were adults and had died of cancer. Two were infants who had died of internal hydrocephalus and meningococcic meningitis respectively. Vascular, valvular and pericardial lesions similar to those present in the acute lupus erythematosus cases were absent in these 4 hearts.

Superficial valvular lesions may be much more extensive than those thus far described. At the same time there are usually also early and milder lesions elsewhere on the same valve or on other valves. Thus, occasionally the pulmonic and tricuspid valves have shown these milder lesions when gross vegetations were visible only on the mitral valve. In the more severe lesions (Fig. 14) the process of degeneration of the surface layers is more

marked and more extensive than the proliferative processes. The verrucae consist of broad areas of degeneration and eosinophilic change partially admixed with fibrin and platelet thrombi. The proliferated cells become swollen and their protoplasm undergoes granular degeneration. The degenerated swollen cells as well as other constituents occasionally present (platelets) form a fused mass which is extruded from the underlying layers and becomes the major portion of the verrucae. When these degenerating cells undergo extensive pyknosis and karyorrhexis, characteristic bodies are formed which stain a deep blue with hematoxylin, and somewhat resemble lime (Fig. 14). The hematoxylin-stained bodies occurring within the granular thrombotic material on the valves and within the valve pockets appear to be pathognomonic of the disease under discussion. As with the eosinophilic coalescent bodies, these hematoxylin-stained bodies are occasionally seen elsewhere in the heart, particularly within blood vessels in which they appear as "blue celled" granular plugs (Fig. 16).

Lesions within the valve leaflets proper appear to be secondary to the changes already described and are generally severe only when extensive lesions are present on the surface of the cusps. On the other hand, the leaflet occasionally presents an almost complete necrosis of most or all of its layers with a minimum of exudate or reaction. A further distinctive feature of the valvular lesions in acute lupus erythematosus (and in atypical verrucous endocarditis) is the characteristic bilaterality of the process, *i.e.* the proximal and distal valvular layers are often equally or simultaneously involved (Fig. 14). Sometimes, indeed, the inflammatory changes within the valve extend throughout its entire breadth.

The character of the valvulitis is also unique. A distinctive feature frequently noted is the presence of young capillaries of the granulation bud type in which the endothelial cells have undergone swelling and proliferation. The lumens of the larger vessels may appear empty or the proliferative endothelial cells may undergo degeneration, desquamate and form granular plugs. The thick muscular vessels so characteristic of rheumatic fever are not observed except in the cases which have been the seat of a previous attack of that disease. The tissue between the blood vessels is infiltrated with numerous cells, the most characteristic of which is the plasma cell. Lymphocytes are rarely observed.

Polymorphonuclear leukocytes are absent except occasionally when extensive necrosis of the valve has occurred. The rarity of these two types of cells is still another distinction between the lesion of rheumatic fever and the lesions in acute lupus erythematosus and "atypical verrucous endocarditis." While the plasma cell is the most characteristic, other types of cells may predominate, particularly fibroblasts, macrophages, large mononuclear cells and binucleated or multinucleated cells, many of which show the swelling and granular degeneration which have been noted in the cells of the surface.

The intensity of the valvulitis is quite variable. In early or mild lesions there is nothing more than a histiocytic proliferation in the distal portion of the cusp and just below the surface (Fig. 13), associated with a diffuse scattering of plasma cells and binucleated cells with abundant eosinophilic cytoplasm. In severe cases, as noted, the valve may undergo marked destructive changes. The whole cusp undergoes a liquefaction necrosis and there is an abundance of necrobiotic "ghost" cells in which only the pyknotic and fragmented nuclei are visible. The granular thrombotic material generally present at the surface may extend into and include the entire width of the cusp, and the hematoxylin-stained bodies may be visible within the cusp proper.

Valvular lesions may be revealed microscopically when the gross appearance of the valves is normal. Thus, in some cases, even in the absence of gross valvular lesions, the microscopic criteria are sufficiently characteristic to make a diagnosis of valvular disease in acute lupus erythematosus. The most distinctive features of the microscopic alterations are: the hematoxylin-stained bodies, necrosis of the valve in the absence of bacteria, widespread multinucleated eosinophilic coalescent bodies, the bilateral involvement of the cusps, and the characteristic valvulitis with plasma cells and young thick granulation bud capillaries. The 5 cases definitely associated with rheumatic lesions presented valvular alterations, some of which could be identified as characteristically rheumatic, while others were similar to those described above. Of the remaining 18 cases, the distinctive features of the valvulitis of acute lupus erythematosus were observed 14 times in the tricuspid valve, 10 times in the mitral valve, 8 times in the pulmonic and 6 times in the aortic. In addition, the valves not

included in these figures occasionally showed lesions that were not considered characteristic of the disease, either because they were mild or because only one or two of the above mentioned features were present. In 15 of the 23 cases the valvular lesions were well defined and characteristic; in 2 others the only valvular lesions present were eosinophilic multinucleated bodies on the endocardium. These findings alone, however, even in the absence of a rheumatic or bacterial process, are insufficient to make a definite diagnosis. In 3 cases the lesions of the disease under discussion were overshadowed on the valves by non-bacterial thrombotic deposits on healed rheumatic processes,[16] but were present elsewhere in the heart. In the remaining 3 cases no appreciable valvular lesions were found.

Pocket Lesions

Verrucae were grossly visible in the valve pockets in several cases. Microscopically the valve pockets frequently showed characteristic evidences of the disease, lesions being present in one or more valve pockets in at least half of the cases. The lesions varied greatly in intensity. Occasionally there was a mild non-specific reduplication consisting of a gelatinous or collagenous tissue with a variable amount of proliferating cells and elastic tissue. Sometimes there was a marked degree of proliferation of endothelial and mononuclear cells forming polypoid structures. When degenerative changes occurred in these cells or when there was considerable vascularization of the pocket region, these polypoid structures assumed a spongy appearance. Not infrequently there were multinucleated eosinophilic coalescent bodies, seemingly extruded from the pocket and identical with those described above.

When the lesions were well developed they were quite characteristic and assumed a granular thrombotic structure apparently identical with those on the valve surface. These lesions have already been described in detail in the report on "atypical verrucous endocarditis" (Gross [10]). In brief, they consisted of a peculiar material which filled the entire valve pocket and often linked the ventricular surface of the auriculoventricular cusps and chordae tendineae intimately with the subjacent ventricular wall. The mass appeared to consist of several components; the ground substance was a very fine granular material staining pink with hema-

toxylin and eosin, yellow with the Van Gieson method, and a very faint orange with Sudan III. Within the granular ground substance there were seen irregular wavy clumps and packets of "hematoxylin-stained bodies." Occasionally there was a third component of fibrin and possibly blood platelet conglutinations. This granular material, like that on the valve surface, was linked to the subjacent structures by the peculiar type of granulation tissue described as occurring within the leaflets themselves. This consisted of numerous capillaries with proliferating endothelial cells encroaching on bloodless lumens, and many plasma and other mononuclear cells which often contained abundant granular cytoplasm. Frequently binucleated and multinucleated cells were present. These cells seemed to be formed by coalescence of the granular mononuclear cells, while the hematoxylin-stained bodies resulted from their rupture and the fusion of their nuclei when they underwent degeneration and necrosis. Various bacterial stains disclosed no organisms in the granular thrombotic mass.

Chordae Tendineae Lesions

Macroscopic verrucae were present on the chordae tendineae of the mitral or tricuspid valves in 7 cases. These were generally seen in the chordae tendineae pockets near their attachment to the valve, at their attachments to the papillary muscle and, occasionally, as an extension from the auricular surface of the valve margins. Microscopic lesions on the chordae were seen in 11 cases. The appearance of the lesions did not differ essentially from those occurring in the valve pockets and in the valves. Most frequently there were vascularized inflamed agglutinations binding the chordae to the ventricular or papillary endocardium. Proliferative, spongy and desquamative lesions, similar to those described, were present. Occasionally there were present granular bluish masses already referred to as hematoxylin-stained bodies. The inflammatory cells and vessels were similar to those described above.

Mural Endocardial Lesions

Ventricular endocardial verrucae were observed grossly in 13 cases. When present they were practically pathognomonic of acute lupus erythematosus (and of atypical verrucous endo-

carditis). In 5, the lesions extended from the posterior mitral pocket to involve the immediately subjacent left ventricular endocardium. In 4, the lesions were situated on the right ventricular aspect of the interventricular septum; in 1 case, at the chordae attachment to the right ventricle; and in 3, on the left ventricular endocardium at the tip of, or behind, the papillary muscles or below the aortic valves. The appearance of the verrucae was similar to that seen on the auricular endocardium, but occasionally reached much greater size.

Histologically the mural endocardial lesions resembled those on the surface of the valve and valve pockets. Granular thrombotic material, proliferative, polypoid and desquamative lesions, and reduplications were present. The granulation tissue was less abundant than in the valvular endocardium, and the blue celled lesions were never as extensive. On the other hand, the papillary muscles, when involved, showed marked infiltration with plasma and mononuclear cells, intense vascularization and granular thrombotic material on its surface.

Pericardial Lesions

Libman and Sacks [1] mentioned pericarditis as a characteristic clinical feature in their cases, while Mook, Weiss and Bromberg,[9] Baehr, Klemperer and Schifrin,[3] and others noted its presence in diffuse lupus erythematosus. Gross pericarditis was observed in 14 of the 23 hearts under discussion. In 4 of these there was a dense, universal adhesive pericarditis; in 5 a diffuse fibrinous exudate with a variable amount of serofibrinous effusion; and in the remaining 5 there was a local fibrous or fibrinous pericarditis usually on the posterior surface of the right auricle.

Microscopic pericardial lesions were present in one or more sections of all of the cases in this series. In 5 cases these lesions were mild and required diligent search to reveal them. In the 5 definite rheumatic cases, and in 2 others, there were certain abnormalities previously noted as occurring in rheumatic heart disease,[17] but even in these cases there were other features similar to those found in the group under discussion. The pericardial lesions to be described were observed 21 times behind the root of the pulmonary artery, 19 times behind the left auricle, 13 times behind the root of the aorta, and 11 times behind the right auricle

in the tricuspid valve section of the standardized blocks. The latter section, however, frequently had little, and occasionally no, pericardial tissue. These statistics do not include a number of cases in which lesions, while present, were either too mild and localized, or so combined with rheumatic lesions as to make the diagnosis of "atypical" pericardial lesions uncertain.

As with the valves, the earliest pericardial changes appeared to involve the lining endothelium. The normally flattened endothelium was converted into high cuboidal cells. In localized areas these were undergoing proliferation, necrosis and desquamation.

As a result of proliferation, polypoid structures were formed in which, in addition to endothelial cells, there were numerous mononuclear cells (Fig. 11). These cells contained rounded vesicular nuclei, or deeply staining solid nuclei with purplish cytoplasm. Occasionally there were binucleated cells with pinkish granular cytoplasm as well as multinucleated cells. Necrotic changes were frequent. Sometimes the polyps were intensely vascularized by a granulation bud type of capillaries. Not infrequently, round or oval multinucleated bodies appeared to have been extruded into the pericardial cavity. These differed somewhat from similar bodies in the pericardium in cases of acute rheumatic pericarditis in the type of cells, those in the latter being essentially desquamated endothelial strips with lymphocytic or polymorphonuclear leukocytes.

While the pericarditis in the cases of acute diffuse lupus erythematosus was not infrequently described grossly as "fibrinous," fibrin was almost invariably absent or of minimal quantity. When present, it usually occurred in the cases considered to be associated with a rheumatic process. Occasionally a granular degenerative ground substance containing necrotic cells was seen at the surface. Sometimes, as a result of proliferation and desquamation, there appeared to be a reduplication of the surface pericardial layer consisting of endothelial and mononuclear cells in a loose connective tissue.

The lamina propria and adipose layers of the pericardium generally revealed characteristic alterations. There were distinctive, newly formed vessels of the granulation capillary bud type (Fig. 15), as well as striking lesions of the vessels normally present in the pericardium. The latter were occasionally partly occluded by

granular plugs or the lumen appeared to have been recanalized by newly formed channels running through the plugs (Fig. 16). The vascular endothelium showed granular swelling, proliferation and desquamation. Polypoid structures somewhat resembling those in the valvular and pericardial surfaces were occasionally observed (Fig. 12). There were also occasional hematoxylin-stained bodies or eosinophilic multinucleated bodies apparently extruded into the lumen, resembling the structures described in connection with the valvular lesions. In many cases the walls of the vessels, particularly of small and medium sized veins, showed a granular necrosis (Fig. 17). This was associated with endothelial swelling, proliferation and desquamation, with granular plugs, and occasionally with a rather characteristic channeling due to septums of fibroblasts running across the lumen. Sometimes the entire wall and its surroundings were infiltrated with cells resembling endothelial cells. In 1 case there was a necrotizing panarteritis similar to that in periarteritis nodosa.

The newly formed vessels were distinctive thick capillaries of the granulation bud type. Unlike the granulation capillaries in rheumatic pericarditis, they were neither congested with red cells, nor assumed the peculiar corkscrew shape so often seen in that disease. The endothelial cells were swollen and actively proliferating. The cell boundaries were indistinct and the cytoplasm scanty, so that the walls of the capillaries frequently appeared as a bluish smudge with numerous crowded nuclei. As a result of proliferation the walls were frequently two or three cells thick, so that the lumen appeared greatly narrowed or even occluded. In such cases the vessels appeared like almost solid bluish cords of cells or, when cut in cross section, like solid circular or oval cellular masses (Fig. 15). In only 2 cases of the 100 non-rheumatic controls were there found capillary lesions of the pericardium which bore some resemblance to those described above. One was from a case with valvulitis and glomerulonephritis and the other was in a case with uremia and non-bacterial thrombotic endocarditis. In both cases other distinctive pathological features of acute lupus erythematosus were absent.

The pericardial layers showed varying degrees of cellular infiltration. These were scattered diffusely or formed compact foci

and were most frequently found in the subendothelium, the fat septums and around blood vessels. As elsewhere in the heart, the distinctive cells were plasma cells, peculiar mononuclear cells and multinucleated cells. The mononuclear cells generally were large and contained a deep, solidly staining nucleus (which was oval, round, kidney-shaped or irregular, and eccentrically located in the cell) and abundant pink granular cytoplasm. Binucleated and multinucleated cells seemed to result from these cells or by coalescence of plasma cells. Sometimes young proliferating fibroblasts were present in abundance. Lymphocytes and polymorphonuclear leukocytes, so typical of rheumatic pericarditis, were generally not conspicuous. In the inactive stages of rheumatic heart disease in which Aschoff bodies are absent, cellular infiltrations are almost uniformly composed of lymphocytes situated in the lamina propria and immediately subjacent fat. When such infiltrations were present in any considerable number in cases of acute lupus erythematosus, we suspected a complicating preceding rheumatic infection, and this was borne out by other pathological findings and by the clinical history and examination. Similarly, the occurrence of considerable fibrin or of congested capillaries led to the same association. When rheumatic lesions were present, however, they could usually be distinguished from the "atypical" pericardial lesions which were present side by side. In cases in which polymorphonuclear leukocytes were found in any considerable degree, their presence could be associated with the terminal bacteremia in these cases. In occasional cases there were foci of fat necrosis or serous atrophy of fat cells. Nearby were gland-like formations composed of young regenerating fat cells (Fig. 18) and usually plasma cell reaction.

Characteristic lesions were observed in the septums of the adipose layer. These septal lesions appeared as signet formations, resulting from accentuation of the septums by cellular proliferation, infiltration and vascularization. The vessels were of the thick granulation capillary type described above, with proliferated endothelial cells and narrowed or occluded lumens. The cells in the thickened septums consisted of large, deeply staining mononuclear cells. Occasionally, however, they appeared to be young proliferating fat cells with a bluish granular cytoplasm.

Myocardial Lesions

Neither clinically nor pathologically was there evidence of severe myocardial disease. Myocardial lesions consisted essentially of vascular alterations. The endothelial cells occasionally appeared high and cuboidal. Proliferation, degeneration and polyp formation were sometimes observed. More characteristically, granular plugs were seen in the lumens of myocardial arterioles and venules, sometimes assuming a lobated form. The eccentric slit-like space representing what was left of the lumen was generally covered by endothelium continuous with the vascular endothelium proper. Occasionally, degenerated cells appeared intermingled with the granular material. These vessels have been termed "granular plugged vessels." An apparently later stage of the same lesions showed partial organization of the plugs with the formation of two or more channels in the lumen by connective tissue septums. These are termed "channeled vessels." [10] Not infrequently, isolated vessels showed hematoxylin-stained bodies or multinucleated eosinophilic bodies similar to those described above. Occasionally these bodies appeared in the interstices of the myocardial bundles (Fig. 9). These various vascular lesions were observed with special frequency in the section through the posterior papillary muscle, but were found almost as often in the various portions of the ventricular and auricular myocardium.

Aside from these lesions there were foci of interstitial inflammation. These consisted of polymorphonuclear leukocytes, lymphocytes, macrophages and plasma cells. Purulent foci with occasional myocardial destruction and fibrosis were usually found in the cases in which a terminal bacteremia had occurred. In the cases with a previous rheumatic infection, lymphocytes and polymorphonuclear leukocytes generally predominated. These cases also showed characteristic interfascicular scars and occasionally vessels with intimal musculo-elastic hyperplastic changes.[18] In the uncomplicated cases of lupus erythematosus the predominating cells were usually plasma cells and the peculiar large mononuclear cells observed elsewhere in the heart. In addition, there were numerous histiocytes and fibroblasts. Such foci of plasma cells and mononuclear cells were occasionally associated with the thick proliferating endothelial bud type of capillaries which have been described

as occurring more frequently in the pericardium and valves. In the sections with extensive granular plugged vessels there were usually minute infarcts in various stages of healing. Aschoff bodies were absent in all cases.

Lesions of the Aorta and Pulmonary Artery

In 2 cases the aortic intima, and in 2 the intima of the pulmonary artery showed proliferative-degenerative lesions similar to those occurring on the valvular and mural endocardium (Fig. 10).

Cardiac Lesions in the Libman-Sacks Syndrome without Lupus Erythematosus

There were 4 cases available in which the clinical and gross pathological lesions were those described by Libman and Sacks, but there were no cutaneous lesions of lupus erythematosus. Two of these were included in the report of these authors. All 4 had unusual valvular vegetations, 2 of them had gross mural endocardial lesions, and all 4 had gross pericarditis. In these respects they did not differ from the cases with lupus erythematosus. In at least 1 case, and in possibly a 2nd, there was significant evidence both clinically and pathologically of rheumatic heart disease.

Microscopically all 4 of the cases revealed the characteristic cardiac lesions described in the above cases with acute lupus erythematosus. The valves showed the bilateral endothelial and polypoid lesions, the hematoxylin-stained bodies and the multinucleated eosinophilic bodies, necrosis, and the characteristic granulation tissue of plasma cells, mononuclear cells and thick granulation bud capillaries. In all 4 of the cases there were found the same pericardial lesions as described in the above 23 cases. The granular, plugged, channeled and necrotic vessels were quite frequently observed in the myocardium and pericardium.

Discussion

Microscopic cardiac lesions were invariably noted in the fatal cases of diffuse lupus erythematosus described above. In 6 cases a definite microscopic diagnosis was not warranted, either because of the mildness of the lesions or the predominance of rheumatic lesions. In 17 of the 23 cases vegetations were noted macroscopically on the valve, valve pocket and/or mural endo-

cardium. In 9 of the 17 cases the vegetations were sufficiently characteristic grossly to make a diagnosis of the disease. In the others they could not be distinguished grossly from rheumatic verrucae or those of non-bacterial thrombotic endocarditis.[16] Essentially there was an endopericarditis, but myocardial vascular lesions were not infrequent. Gross verrucous lesions of the valvular and mural endocardium, and gross pericarditis were often observed when the lesions were extensive, but even in the absence of gross abnormalities, microscopic examination often revealed distinctive cardiac lesions.

Taken as a whole, these lesions could be readily distinguished from those observed in other cardiac diseases, and some were sufficiently characteristic to permit a diagnosis of the disease under discussion on the basis of the microscopy of the heart alone. Gross lesions were sometimes absent and sometimes indistinguishable from rheumatic heart disease, non-bacterial thrombotic endocarditis or bacterial endocarditis. The latter, however, could easily be eliminated by the absence of bacteria in the vegetations and by microscopic examination to eliminate the bacteria-free stage of that disease. The pericarditis was indistinguishable grossly from other forms of fibrinous, serofibrinous or adhesive pericarditis. Occasionally the gross appearance and location of the valvular lesions were sufficiently distinctive to permit a diagnosis of "atypical verrucous endocarditis." This was possible when the verrucae were of the "flat spreading variety" and occurred on the auricular or ventricular endocardium, on the papillary muscles or chordae tendineae, or when they occurred conspicuously in the valve pockets and on the ventricular surface of the auriculoventricular valves or arterial surfaces of the semilunar valves. The diagnosis could be suspected also if the verrucae occurred solely or predominantly on the valves of the right side of the heart, or if they were present on several valves, including the pulmonic valve but not the aortic.

Except for the 6 cases previously mentioned, the microscopic findings in the heart usually provided more rigid, well defined criteria for diagnosis. While characteristic lesions were generally present in many portions of the heart, in occasional cases definitely distinctive lesions were limited to only one portion of the heart. Usually, most or all of the distinctive features described

above were observed in any one case, but sometimes the microscopic diagnosis rested on a few of the more characteristic findings. Even in the 6 cases in which a definite microscopic diagnosis of this disease was not warranted, evidence existed which indicated the presence of endocardial, pericardial or myocardial irritation which, however, had not as yet been sufficiently intense to produce more than mild pathological alterations.

It is difficult to speak of specific lesions on the basis of a comparatively small series of cases, yet some of the microscopic lesions appeared so characteristic of the disease as almost to justify that term. The hematoxylin-stained granular bodies found especially frequently in the valves and valve pockets have been thus far encountered only in this disease. The milder, spongy, superficial valvular lesions containing degenerating, ghost-like mononuclear cells are probably earlier stages of the hematoxylin-stained bodies and almost as characteristic. Although structures resembling or identical with eosinophilic bodies are occasionally observed in active rheumatic or bacterial valvular disease, as well as in other conditions, their frequency, and particularly their widespread distribution in this disease (valvular, pericardial, mural endocardial and vascular endothelial surfaces), appear to be of considerable diagnostic importance. Certain other features, while not individually conclusive, permit diagnosis when present collectively. Thus, the occurrence of severe lesions in the valve pockets, bilateral disease of the valve, especially with necrosis and a typical peculiar granulation tissue, form a unique picture. In the pericardium the association of proliferation and necrosis of endothelium with peculiar polypoid formation, multinucleated eosinophilic bodies, cord-like granulation capillaries, infiltrations of plasma cells, mononuclear cells, binucleated and multinucleated cells, fatty lesions of the septums and peculiar vascular lesions also produce a distinctive picture.

Except for 5 of the cases in which there was adequate evidence to believe that a previous attack of rheumatic heart disease had occurred, the pathological lesions were quite different from those of rheumatic disease. Even in the 5 rheumatic cases, the characteristic lesions of the syndrome studied were usually present side by side with those that could be ascribed to rheumatic fever. In these cases the probability of rheumatic disease was postulated

on the microscopic findings, and this was confirmed by the clinical history and the presence of gross rheumatic auricular lesions and/or mitral stenosis with cardiac enlargement. The heart was not enlarged in the uncomplicated cases of lupus erythematosus. Some of the essential microscopic differences from rheumatic fever are the following:

1. Despite the acute febrile clinical course, Aschoff bodies were invariably absent.
2. Certain auricular and valvular endocardial reduplications and palisade formations peculiar to rheumatic fever were absent in the uncomplicated cases.
3. Ring lesions were frequently absent when other portions of the valve were involved. The ring lesions when present differed from those of rheumatic fever in the presence of plasma, mononuclear and multinucleated cells, and thick proliferating granulation capillaries. Thick muscular vessels were almost invariably absent.
4. Valvular disease occurred on either or both surfaces of the valves instead of being confined entirely or predominantly to the auricular surface of the auriculoventricular valves and the ventricular surface of the semilunar valves as in rheumatic fever.
5. There was a marked tendency to valvular necrosis.
6. Valvular verrucae were present occasionally without concomitant evidences of present or past valvulitis.
7. Verrucous lesions occurred frequently in the valve and chordae pockets, and on the endocardium of the ventricles, auricles, papillary muscles and chordae tendineae.
8. Peculiar, granular hematoxylin-stained bodies were present in the valves, valve pockets and blood vessels.
9. The pericardial lesions were associated with little or no fibrin exudation.
10. Pericardial granulation tissue showed frequent plasma, mononuclear and multinucleated cells and proliferating granulation capillaries, but few or no lymphocytes, polymorphonuclear leukocytes or congested capillaries.
11. There were peculiar signet formations in the pericardium due to cellular and vascular proliferation of the septums.
12. There were frequent vascular lesions in the pericardium and

myocardium which are rarely observed in rheumatic heart disease. On the other hand, typical rheumatic vascular lesions such as the intimal musculo-elastic hyperplastic type of proliferation, were absent in these cases.

13. Eosinophilic multinucleated coalescent bodies were frequently present on the valvular, mural, vascular and pericardial endothelium in the absence of rheumatic stigmata and of bacterial endocarditis.

While the microscopic features in the hearts in these cases of diffuse lupus erythematosus differed from those of rheumatic heart disease, they were identical with those in the cases of Libman-Sacks disease (without lupus erythematosus) which showed grossly an "atypical verrucous endocarditis." I commented at the outset on the marked similarity between the clinical features of Libman-Sacks disease and those of acute disseminated lupus erythematosus. I also pointed out that a peculiar form of gross verrucous valvular and mural endocarditis occurred in both diseases, whether the lupus erythematosus skin lesions were present or not. Finally, I have observed that cardiac lesions are invariably present microscopically in both groups of cases and that in the majority of instances these are quite characteristic. Therefore, the conclusion that both groups of cases are essentially examples of the same disease appears cogent.

This conclusion leads to the question of a proper terminology to include both groups of cases. The invariable occurrence of cardiac lesions might lead one to suppose that we are dealing essentially with a cardiac disease. But this supposition is weakened by a consideration of the clinical features of the disease and by pathological observations in other organs. As reported by Libman and Sacks,[1] and by Baehr, Klemperer and Schifrin,[3] and others, the clinical features include besides non-specific features of a general infection, serous membrane involvement including arthritis, pericarditis and pleuritis, renal symptoms, cerebral and meningitic symptoms, disturbances of the formed elements of the blood, purpura and other cutaneous lesions. Pathologically, also, abnormalities are not confined to the heart, for vascular lesions have been described in many other organs, especially the kidneys. On the other hand, we cannot speak of these cases as constituting an essential vascular disease because vascular lesions are not in-

variably present and are often confined to the kidney alone. Thus, in the 18 cases of Baehr, Klemperer and Schifrin [3] in which the hearts were available for study, the occurrence of vascular lesions as tabulated for us by the authors was as follows: In 2 cases there were no vascular lesions; in 8, they were confined to the kidney, 5 of these showing lesions of the glomeruli only, the other 3 showing interstitial vascular lesions as well; in 7, one or more organs besides the kidney were involved, only 4 of them showing diffuse, extensive lesions, and in 1, in which the kidney was not available for study, vascular lesions were found in the ovary and mesenteric lymph nodes. In my studies, 5 of the 8 cases in which the lesions were considered to be confined to the kidney revealed vascular alterations in the myocardium of the type previously described by me.[10] It is of some interest that of the 4 cases with diffuse lesions, 2 individuals had definitely suffered from rheumatic heart disease, 1 had a history of rheumatic endocarditis at the age of 10, and 1, with periarteritis nodosa, showed a grossly vascularized mitral valve and early mitral stenosis. This would suggest the possibility that extensive diffuse vascular lesions may occur more readily in this disease only when some other disease which also attacks blood vessels had previously been present.

An etiological terminology is at present impossible because the etiology is unknown. The use of "atypical verrucous endocarditis" as a term for the entire disease is undesirable because: (1) the unusual gross lesions to which it applies are frequently absent; (2) it fails to include the very characteristic microscopic lesions both on the endocardium and elsewhere in the heart; (3) it fails to consider the occurrence of vascular lesions in other organs; (4) it places undue emphasis on the heart in a disease which the original authors themselves described as a general one with protean manifestations. The term "atypical verrucous endocarditis" should be reserved purely for the pathological finding of peculiar verrucous lesions on the valvular and mural endocardium. It is equally objectionable to refer to the general disease as lupus erythematosus because this refers to a symptom not invariably present, and because it places undue emphasis on the skin manifestation as such, which while admittedly dramatic in appearance is not as significant as the lesions in various internal organs. The

term "diffuse vascular disease" appears inadequate because: (1) it may refer equally to rheumatic fever, periarteritis nodosa and certain infectious diseases; (2) vascular lesions are not invariably present; and (3) when present, they are not always diffuse but generally confined to the kidney or kidney glomerulus.

Until an etiological nomenclature is possible, I prefer to group these cases both with and without lupus erythematosus under the term, "Libman-Sacks disease." While the title of the report of these authors stressed the endocardial lesions, its contents emphasized the clinical syndrome, the description of which is remarkably complete. Their cases first demonstrated the occurrence of the general disease both with and without the skin lesions of lupus erythematosus. They first presented characteristic pathological changes in the heart, of which further microscopic studies have provided distinctive pathological diagnostic criteria of the disease. It was through the segregation of these authors' original 4 and additional cases of non-rheumatic endocarditis that Baehr and his co-workers observed the frequent occurrence of characteristic vascular lesions in the kidneys and other organs. Aside from giving due credit to the original observers, the use of the term "Libman-Sacks disease" has the advantage of emphasizing a general clinical picture. It does not lay undue stress on a skin lesion, on peculiar gross endocardial lesions, or on vascular lesions which may or may not be present and may or may not constitute the essential feature of the disease.

In a previous communication [16] we followed the classification of Libman [19] in listing the endocardial lesions of this disease (atypical verrucous endocarditis) in the category of "indeterminate endocarditis." This was largely done because the existence of the disease as an entity and its relation to other diseases were not as yet sharply defined. In view of the facts presented in this discussion concerning the characteristic clinical features and pathological findings, this disease should now be taken out of the "indeterminate" category and listed as a separate entity as is done with rheumatic fever.

Further comment as to the relationship between Libman-Sacks disease and lupus erythematosus appears pertinent. As regards the form of the disease with disseminated lesions in which there is an acute febrile and fatal course, our studies would indicate that

all such cases belong in the category of Libman-Sacks disease, and that all of them show characteristic lesions in the heart and perhaps in other organs. The relationship between Libman-Sacks disease and cases of the chronic localized and disseminated lupus erythematosus which run a benign course is not clear. There is no definite agreement among dermatologists as to whether the acute and fatal forms of lupus erythematosus represent a different disease or different stages or manifestations of the same disease. The clinical symptoms and course are markedly different. Nevertheless, in at least 2 of the cases the onset of lupus erythematosus occurred 2 or 3 years before the final illness, which was of the acute disseminated form, and there were asymptomatic intervals of considerable duration. But even in these cases there appeared to be some distinctions from the chronic discoid form, even when the lesions were localized. First, there was no evidence of the scarring, and little or no atrophy which is so typical of the benign discoid form. Secondly, even with the original cutaneous outbreaks which disappeared, there were some constitutional symptoms such as fever and multiple arthritis. This would tend to indicate that Libman-Sacks disease may run a more prolonged and chronic course than would appear from observing the acute fatal illness. Unfortunately, no autopsy material was available for study from benign or localized forms of the disease. It is interesting in this connection that Libman and Sacks suggested that this disease may occur in mild form and with recovery.

A final word seems necessary in regard to the possible relationship between Libman-Sacks disease and rheumatic fever. The clinical differences have been pointed out by Libman and Sacks, and the pathological differences in the cardiac lesions by these authors and by the writers. Nevertheless, it is important to indicate certain striking resemblances. The clinical resemblances need not be considered in detail in this paper except to point out that the acute febrile course with negative blood cultures, and the occurrence of polyarthritis, pericarditis and cardiac murmurs usually lead to a diagnosis of rheumatic fever instead of Libman-Sacks disease. Pathologically, also, there are certain resemblances as well as dissimilarities. The resemblances consist essentially in the marked tendency of both diseases to affect endothelial-lined structures, namely, pericardium, endocardium, joints and blood

vessels. Considering the lesions in the heart, which is invariably or almost invariably affected in both diseases, this similarity appears to be a superficial one. In rheumatic heart disease the primary and fundamental injury involves the collagen. This is true in the endocardium, the pericardium and myocardium. The endothelial changes on the surface and the formation of verrucae are secondary to the deeper valvulitis associated with collagen swelling and degeneration. The disease begins in the valve ring [14] and spreads by contiguity toward the valve tip.[15] In Libman-Sacks disease the primary and fundamental change appears to be in the endothelium. Thus, there is generally superficial proliferation, necrosis and verruca formation before there is a deeper valvulitis, the latter representing a reaction to the alterations on the surface. The valve ring may be entirely normal if the valve changes are not sufficiently extensive to reach this region. Furthermore, because of the fundamental nature of the endothelial change, the verrucae are more extensive ("spreading"), involve both valve surfaces, the valve pockets, the mural endocardium and that of the papillary muscles and chordae tendineae, in fact, all endothelial surfaces.

A similar distinction between the two diseases is seen in the pericardial lesions. In rheumatic fever the collagen of the lamina propria is primarily injured and the overlying endothelium reacts by exudation. Proliferation, desquamation and degeneration are secondary. In Libman-Sacks disease the proliferation and necrosis of the surface endothelium are primary, exudation is minimal and the subjacent alterations are reparative reactions to the injury at the surface. Finally, the myocardial lesions in rheumatic fever are of primary significance because of the extensive vulnerable collagenous interstitial tissue. In Libman-Sacks disease the lesions observed vary greatly with the extent of injury to the vascular endothelium and, except in rare instances where there is considerable closure of vessels by plugs with consequent minute infarctions, there is no significant injury to the myocardium proper, and myocardial failure is not a clinical feature of the disease.

The presence of presumptive rheumatic heart disease in at least 5 of the 23 cases of acute lupus erythematosus is worthy of note. While Aschoff bodies were not found in these cases, that diagnosis was substantiated by enlargement of the left auricle and pulmonary

conus, the typical gross rheumatic auricular lesions, the mitral stenotic valvular deformity and the numerous characteristic rheumatic microscopic lesions. The association of these two otherwise distinctly different diseases suggests the possibility that Libman-Sacks disease, like subacute bacterial endocarditis, is perhaps more apt to occur in patients in whom rheumatic fever had previously injured cardiac and vascular structures.

The complete clinical picture of this disease has been described by Libman and Sacks, in whose communication the details can be found. However, the following combinations of clinical features may be mentioned as clues to the diagnosis which can be made ante mortem:

1. Disseminated lupus erythematosus with persistent fever and constitutional symptoms, arthritis, renal symptoms, pericarditis, and so on.
2. Cases resembling rheumatic fever associated with acute pericarditis and white-centered petechiae.
3. Cases resembling rheumatic fever in which there is evidence of acute glomerulonephritis and azotemia, especially with normal blood pressure.
4. Cases resembling rheumatic fever associated with thrombocytopenic purpura.
5. Cases resembling rheumatic fever in which there is leukopenia despite persistent fever.
6. Cases with prolonged fever, azotemia and uremia in which the blood pressure is normal.
7. Cases resembling subacute bacterial endocarditis in which blood cultures are persistently negative and there are no gross embolic features.
8. Cases resembling rheumatic fever or subacute bacterial endocarditis in which protracted or recurrent pleural and pericardial signs are present without evidence of organic valvular lesions.

There are no clinical diagnostic tests by which the diagnosis can be corroborated. Since, however, these cases have thus far all proved to be fatal, confirmation of the diagnosis can be made postmortem by the various macroscopic and microscopic lesions previously described,[10, 3] and by those described in this report.

Summary

A study has been made of the hearts from 23 fatal cases of disseminated lupus erythematosus. The lesions observed were compared with the cardiac lesions in 4 cases of "atypical verrucous endocarditis" without lupus erythematosus. The characteristic macroscopic valvular and mural endocardial lesions of "atypical verrucous endocarditis" were observed in at least 8 cases of lupus erythematosus. Moreover, microscopic lesions were observed in all of the 23 cases, particularly in the valve rings, valve leaflets, valve pockets, mural endocardium and pericardium. With few exceptions, these lesions were characteristic of the disease and many were identical with those observed in the 4 cases without lupus erythematosus.

In a discussion of terminology, it was pointed out that the pathological lesions were not confined to the heart and might involve other organs, particularly the vessels of the kidney. On the other hand, these vascular lesions in other organs are sometimes mild and occasionally absent. Furthermore, it is possible that the clinical syndrome may occur without demonstrable cardiac lesions, although macroscopic or microscopic alterations were invariably present in this series. The common denominator appears to be the clinical features described by Libman and Sacks, the cutaneous manifestations of diffuse lupus erythematosus being frequently but not invariably present. It is, therefore, suggested that the two groups of cases which have been previously called acute disseminated lupus erythematosus and atypical verrucous endocarditis, respectively, should be placed into the single category of Libman-Sacks disease.

Criteria are described for the pathological diagnosis of Libman-Sacks disease on the basis of cardiac lesions, and these are distinguished from the lesions of rheumatic fever and subacute bacterial endocarditis.

NOTE: I wish to thank Dr. Charles K. Friedberg for valuable assistance.

This paper was completed by Doctor Gross in the fall of 1936. The studies upon which it is based were carried out at The Mount Sinai Hospital, New York, where he was Director of Laboratories. He died in an aeroplane accident on October 17th, 1937.

Between 1936 and 1937 Doctor Gross accumulated a number of new observations which he intended to incorporate in the paper along with a discussion of papers of value that had appeared during that period. He had in mind other additions and changes, largely of a historical nature, related particularly to the development of our recent knowledge of the vascular lesions that may occur in the disease under discussion. The submission of the paper has been delayed in the hope of finding any revised copy of the paper containing these changes. A search of his voluminous notes and records revealed numerous references to the changes that he intended to make. However, a revised copy of the paper could not be found and therefore it was believed that the paper should be published in this form.

EMANUEL LIBMAN

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DESCRIPTION OF PLATES

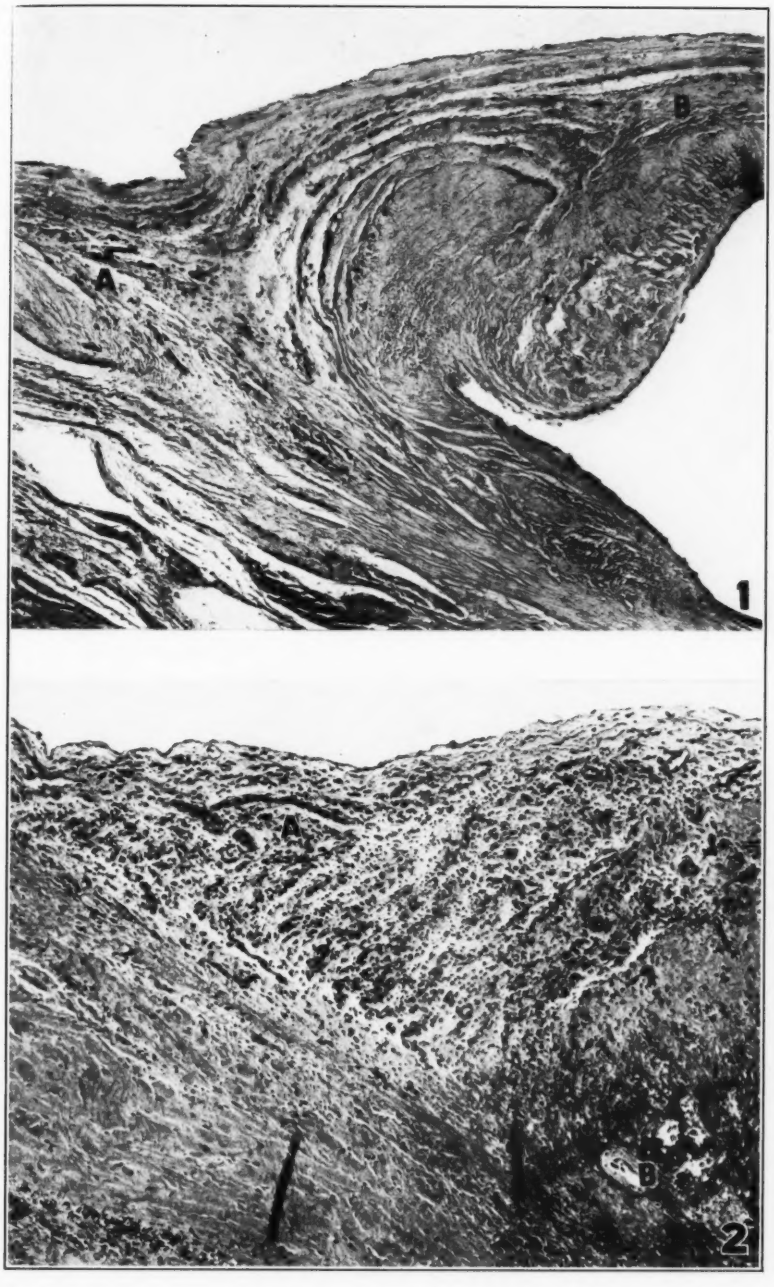
PLATE 77

FIG. 1. Cross section of the posterior mitral valve leaflet from a case of acute lupus erythematosus. Age 19 years. Hematoxylin and eosin stain. Medium power.

A = mild ring lesion. Note young granulation tissue capillaries and rather sparse scattering of plasma cells and large mononuclear cells. B = valve leaflet.

FIG. 2. Cross section of the posterior mitral valve ring from a case of acute lupus erythematosus. Age 10½ years. Hematoxylin and eosin stain. Medium power.

A = active ring lesion. Note the hypercapillarization and presence of thick granulation bud capillaries surrounded by plasma cells, large mononuclear cells and occasional binucleated cells. B = necrotic material in valve ring.



Gross

Cardiac Lesions in Libman-Sacks Disease

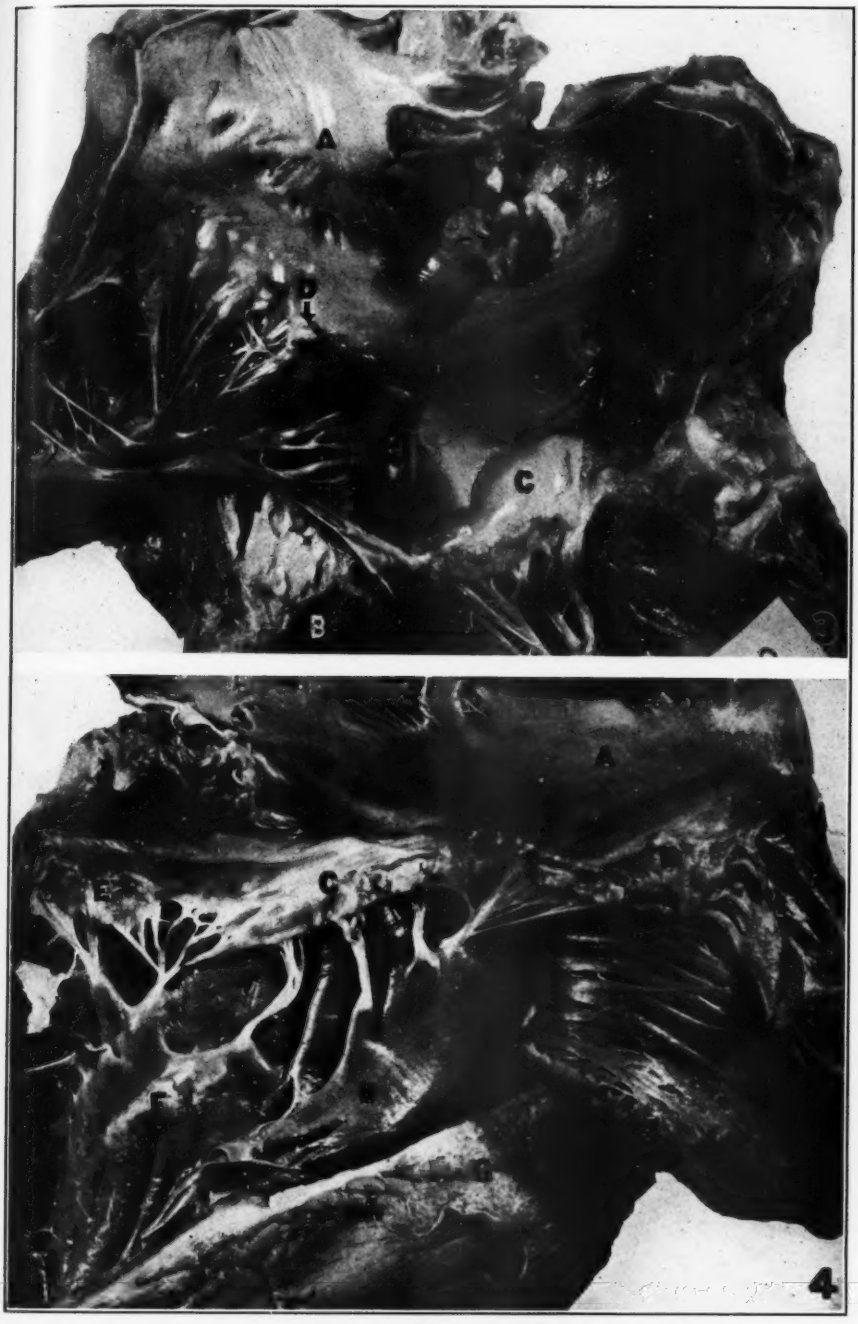
PLATE 78

FIG. 3. Gross photograph of the mitral valve from a case of acute lupus erythematosus showing characteristic lesions of atypical verrucous endocarditis. Age 33 years.

A = left auricle; B = interventricular septum; C = anterior mitral leaflet. Note pyramidal ridge type of verrucous lesion on closure line. D = posterior mitral leaflet showing a larger thrombotic verrucous mass.

FIG. 4. Gross photograph of the tricuspid valve from the case illustrated in Figure 3.

A = right auricle; B = interventricular septum; C = septal leaflet with the flat spreading type of verrucous lesion; D = anterior leaflet with the flat spreading type of verrucous lesion; E = posterior leaflet with a less conspicuous, flat, spreading verrucous lesion; F = typical mural endocardial lesion; G = fresh pericarditis on ventricular surface.



Gross

Cardiac Lesions in Libman-Sacks Disease

PLATE 79

FIG. 5. Cross section of the pulmonary cusp from a case of acute lupus erythematosus. Age 10½ years. Hematoxylin and eosin stain. Medium power.

A = ventricularis layer which has undergone endothelial proliferation with subsequent necrosis. B = fibrosa layer with rare plasma and large mononuclear cells.

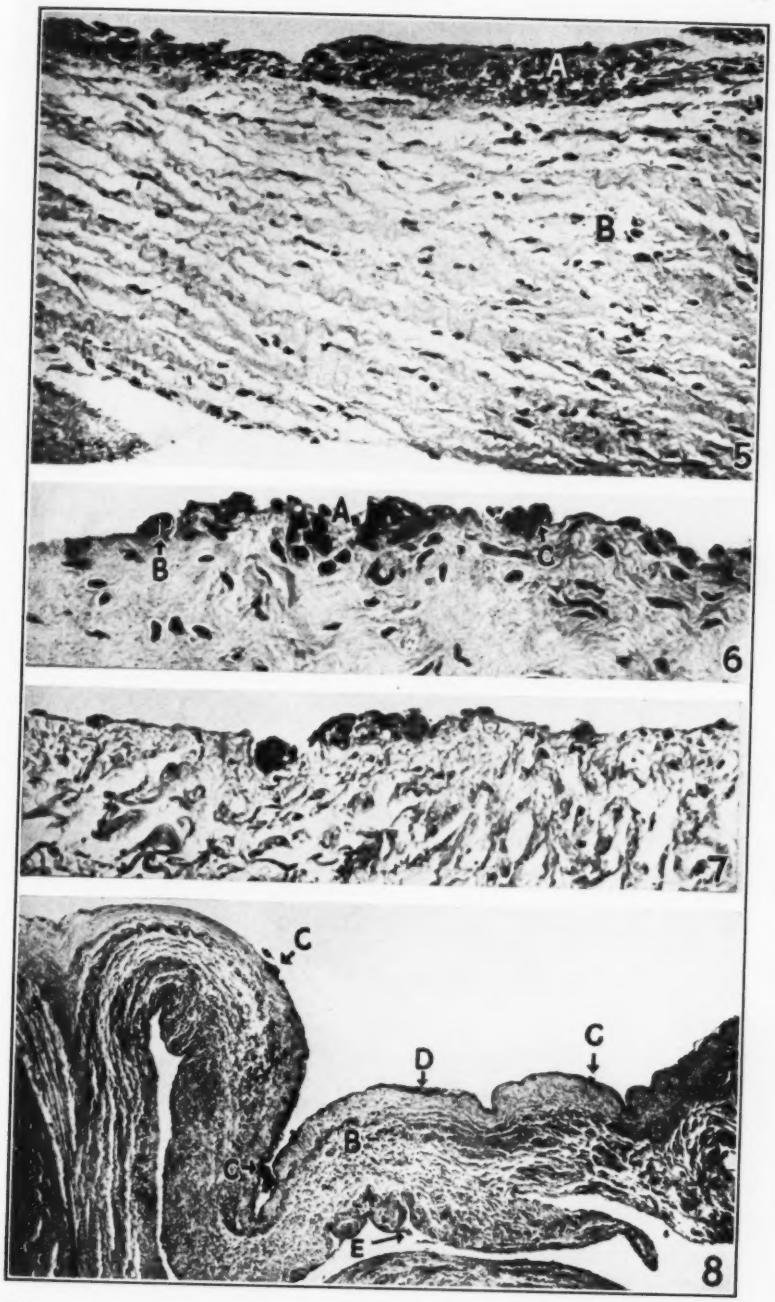
FIG. 6. Cross section of the anterior mitral leaflet from a case of acute lupus erythematosus. Age 18 years. Hematoxylin and eosin stain. High power.

A = proliferated endothelial cells; B = swollen proliferated endothelial cells showing beginning polyp formation; C = early stage in formation of eosinophilic multinucleated coalescent bodies from swollen proliferated endothelial cells.

FIG. 7. Cross section of the pulmonary cusp from a case of acute lupus erythematosus showing eosinophilic multinucleated coalescent bodies on the arterialis surface. Age 18 years. Hematoxylin and eosin stain. Medium power.

FIG. 8. Cross section of the tricuspid leaflet from a case of acute lupus erythematosus showing eosinophilic multinucleated coalescent bodies on auricularis surface. Age 30 years. Hematoxylin and eosin stain. Low power.

A = mild ring lesion; B = scattering of plasma cells and mononuclear cells in spongiosa and fibrosa layers; C = eosinophilic multinucleated coalescent bodies on auricularis surface of leaflet; D = early proliferation and necrosis of endothelial layer on auricularis surface; E = proliferation and stripping of endothelial cells on ventricularis surface.



Gross

Cardiac Lesions in Libman-Sacks Disease

PLATE 80

FIG. 9. Cross section of the posterior papillary muscle from a case of acute lupus erythematosus showing eosinophilic multinucleated coalescent bodies on the mural endocardium between the muscle columns. Age 18 years. Hematoxylin and eosin stain. High power.

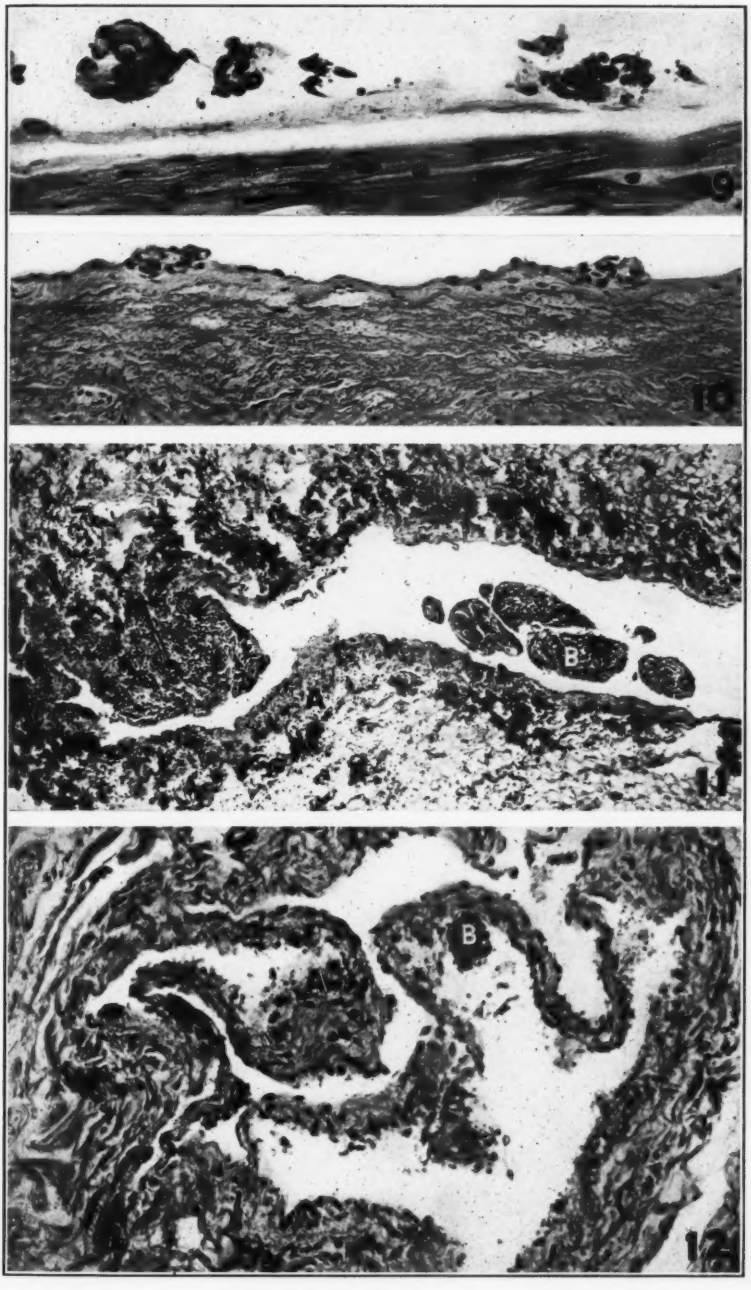
FIG. 10. Intima of the pulmonary artery from a case of acute lupus erythematosus showing formation of eosinophilic multinucleated coalescent bodies. Age 18 years. Hematoxylin and eosin stain. Medium power.

FIG. 11. Pericardial lesion behind the root of the pulmonary artery in a case of acute lupus erythematosus. Age 18 years. Hematoxylin and eosin stain. Low power.

A = cellular infiltration with marked concentration around the lamina propria; B = inflamed pericardial polypi.

FIG. 12. Vein in the left auricular pericardium from a case of acute lupus erythematosus. Age 18 years. Hematoxylin and eosin stain. High power.

A = proliferation and necrosis of the endothelium on the surface of the venous valves; B = eosinophilic multinucleated coalescent body.



Gross Cardiac Lesions in Libman-Sacks Disease

PLATE 81

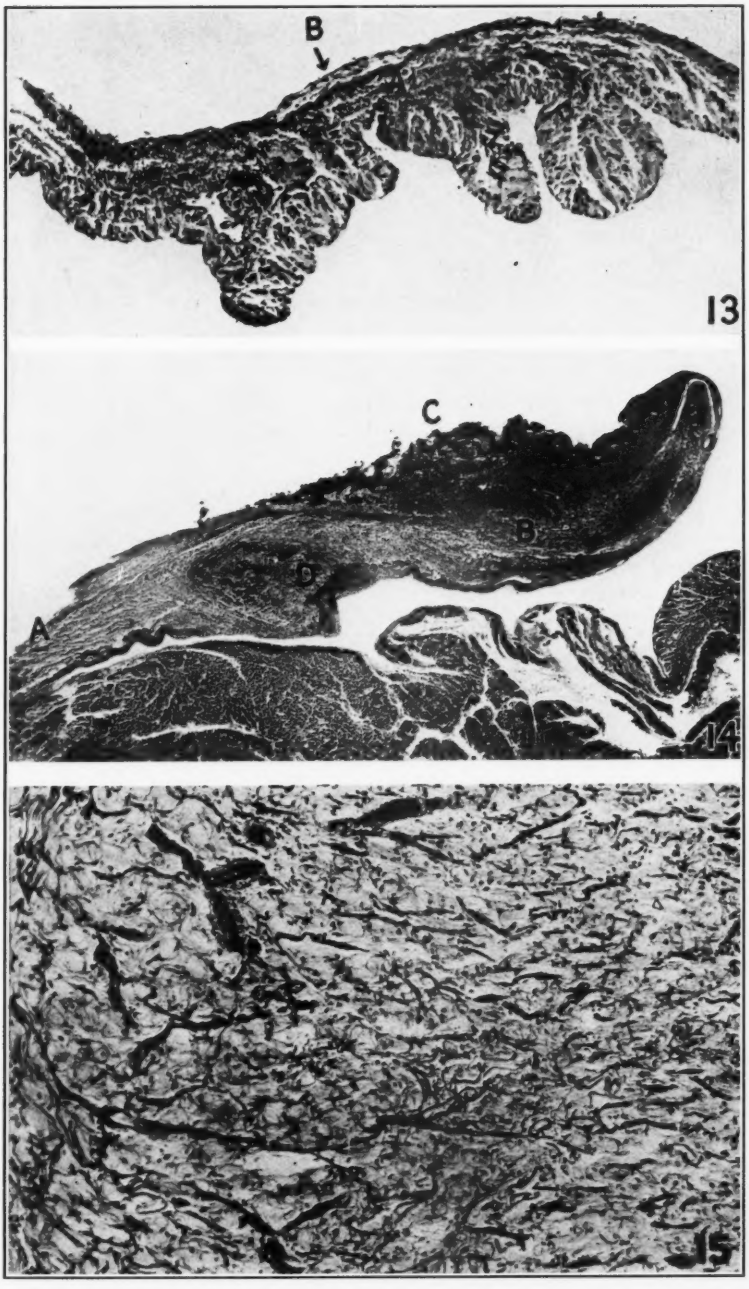
FIG. 13. Cross section of the tricuspid leaflet from a case of acute lupus erythematosus. Age 26 years. Hematoxylin and eosin stain. Low power.

A = mild interstitial valvulitis consisting of scattered plasma cells and large mononuclear cells; B = early edematous reduplication of auricularis layer.

FIG. 14. Cross section of the tricuspid leaflet from a case of acute lupus erythematosus. Age 22 years. Hematoxylin and eosin stain. Low power.

A = intact valve ring; B = necrosis of the valve leaflet; C = massive thrombotic verrucous lesion with hematoxylin-stained bodies on the auricularis surface; D = massive thrombotic verrucous lesion on ventricularis surface.

FIG. 15. Pericardial mantle over the right ventricle from a case of acute lupus erythematosus. Note the numerous granulation tissue capillary buds, some almost completely obliterated by intimal proliferation. Note also scattering of mononuclear cells and thick fat cell septums. Age 26 years. Hematoxylin and eosin stain. Low power.



Gross Cardiac Lesions in Libman-Sacks Disease

PLATE 82

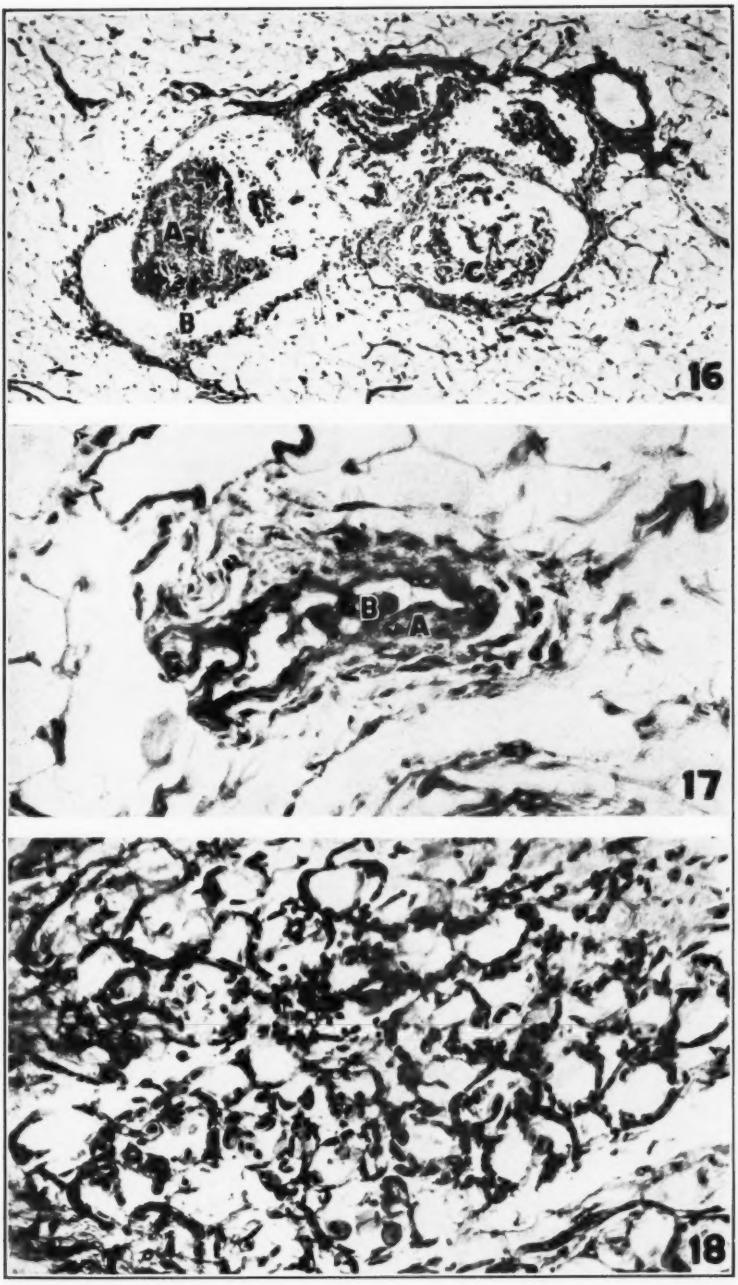
FIG. 16. Characteristic lesion in veins of the left auricular pericardium from a case of acute lupus erythematosus. Age 37 years. Hematoxylin and eosin stain. Low power.

A = granular plug containing hematoxylin-stained body (B); C = channel formation by septums of fibroblasts. Note the raised necrotic endothelium.

FIG. 17. Necrotic vein in the pericardium from a case of acute lupus erythematosus. Age 17 years. Hematoxylin and eosin stain. High power.

A = necrotic proliferated wall of the vein; B = formation of polyp due to proliferated endothelial cells.

FIG. 18. Signet formation in the pericardium of the left ventricle from a case of acute lupus erythematosus. Note the marked proliferation of cells around the fat septums. Age 26 years. Hematoxylin and eosin stain. Medium power.



Gross Cardiac Lesions in Libman-Sacks Disease

HISTOLOGICAL CHANGES IN THE ISCHEMIC KIDNEY*

WITH SPECIAL REFERENCE TO THE JUXTAGLOMERULAR APPARATUS

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Since the discovery by Goldblatt [1] that persistent elevation of blood pressure may be induced by renal ischemia, there has been extensive investigation of the mechanism responsible for the hypertension, as well as a search for some humoral pressor factor or substance. The site of origin of this substance is unknown, although some investigators [2-5] have suggested that it may be a product of the ischemic kidney or formed in the blood flowing through the ischemic kidney, or, if formed elsewhere, retained by the kidney under the conditions of ischemia experimentally produced. Because there may be no demonstrable interference with the excretory functions of the ischemic kidney, it is unlikely that the vasopressor effect is due to a defect of glomerular filtration.

It has been demonstrated that at the vascular pole of the renal glomerulus, in the angle between the afferent and efferent arterioles, there exists a group ("Polkissen") of cells of special structure forming part of a juxtaglomerular apparatus.† These cells, which have been discussed by Ruyter,[6] Oberling,[7] Goormaghtigh,[8] and Spanner,[9] resemble those of smooth muscle, except for their lack of myofibrils. Cells of similar appearance are seen in the cutaneous myoarterial glomus (Spanner,[9] Masson,[10] Schumacher,[11] Clara,[12, 13] and Popoff [14]), in the glomic structures of the carotid body and the cardioaortic zone (Goormaghtigh and Pannier [15]), and in arteries and arterioles elsewhere (Goormaghtigh [16] and Spanner [9]). Characteristic pathological changes occur in these cells of animals with experimental hypervitaminosis D_2 (Goormaghtigh and Handovsky [17]), and in human beings with scarlet fever (Goormaghtigh [8]).

This report presents a description of the changes seen in these cells in dogs and rabbits in which persistent hypertension had been

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† The juxtaglomerular apparatus is in fact composed of two distinct cell groups, one of which is not mentioned in this paper.

induced by renal ischemia. A more detailed report on the cytology of these cells in the kidney of the rabbit has been published.[18]

Methods and Materials

The kidneys of 12 dogs in which experimental hypertension had existed for periods ranging from 24 hours to 17 months were removed, and portions of the organs were fixed in Bouin-Hollande's fluid or in Zenker-formalin. Serial paraffin sections stained by the trichrome method of Masson were made in numbers sufficient to reconstruct the course of an interlobular artery with its terminal arterioles and glomeruli (Fig. 1).

Moderate constriction of the left renal artery (method of Drury [4]) was effected in 3 young rabbits weighing 500 gm. After 3 weeks their kidneys were removed, fixed, sectioned and stained in the same manner as those of the hypertensive dogs. Control observations were made on similarly treated tissue from the kidneys of normal dogs and rabbits.

I am grateful to my colleagues of the J. F. Heymans Institute (C. Heymans, J. Bouckaert, K. Grimson and A. Samaan), and to my former associate, L. Elaut, for ischemic kidneys of dogs. The kidneys made ischemic with externally adjustable clamps (Grimson [19]) were especially valuable. A tabulation of this material is shown in Table I.

Observations

Dogs: After 24 hours of renal ischemia the cells of the juxtaglomerular apparatus are always enlarged and increased in number, and bulge into the glomerulus. After 8 to 17 months of renal ischemia the intraglomerular protrusion is less prominent, but granules have appeared in the afibrillar cells and there is intercellular lipoid infiltration, fibrosis and hyalinization.

The changes in the juxtaglomerular apparatus are accompanied by others in the glomerulus, in the afferent arteriole, in the interlobular arteriole and in the cells of the intercalated segment,* which is anatomically in contact with the juxtaglomerular apparatus.[20, 21, 22] In the afferent arteriole the afibrillar cells may become large and reduce the lumen of the vessel.

* By intercalated segment is meant the distal part of the tubule contortus II (*cf.* G. C. Huber, in Special Cytology, Cowdry, E. V., 1928, 1, 686, lines 28, 29, 30).

Some glomeruli become smaller and many probably disappear. Glomerular hyalinization is seen. In one instance, of the 35 glomeruli counted, 9 were atrophic (but not sclerotic). The others were moderately enlarged. Fibrous adhesions between the glomerular tuft and Bowman's capsule sometimes appear. The vascular pole of the glomerulus is always enlarged and contains more cells than normal, especially in the early stage of glomerular

TABLE I

Animal number	Weight	Duration of renal ischemia		Systolic blood pressure	
		Right kidney	Left kidney	Before renal ischemia	During renal ischemia
	kg.			*mm. Hg.*	*mm. Hg.*
26139	10	10 days	10 days	120	188 170
7239	6	8 days	8 days	144	216
17239	10	24 days	24 days	110	170
24239	12	24 hrs.	24 hrs.	...	...
27339	7	14 days	3 days	...	208 204
28339	9	24 days	7 days	146	238
30339	12	8 days	22 days	140	176
18439	10	21 days	25 days	120	190
10539	17	15 days	7 days	...	206
23539	8	8 days	15 days	...	200
101138	6	Nephrectomy	8 mos.	154	182 206 200
21635	8	Nephrectomy	17 mos.	135	200 204

atrophy; the increased cellularity is still observed 17 months after clamping the renal artery.

In the primary divisions of the renal artery and in the interlobar artery, some of the medial smooth muscle cells become atrophic; others lose their myofibrils and often show active nuclear mitosis. In brief experiments edema, fluid and lipoid material appear in the intercellular spaces. In long experiments collagen is deposited in the intima and superficial media. These changes lead to the formation of fibrotic plaques in positions where medial smooth muscle cells have lost their myofibrils.

In the interlobular prearterioles, especially at their bifurcations,

large, multinuclear afibrillar cells appear and replace almost all the medial smooth muscle cells (brief experiments).

Rabbits: Renal ischemia in the rabbit induces the appearance in every juxtaglomerular apparatus of many large afibrillar cells, and most of these cells acquire cytoplasmic granules which normally are found only in the superficial layer of the cortex. Although in the rabbit most of the changes are found in the juxtaglomerular apparatus, a few granulated cells of the same type appear in the interlobar and interlobular prearterioles and in the afferent arteriole to the glomerulus.

In the intercalated segment the epithelial cells show vacuolization, and lipoid material accumulates between cells. The intercalated segment disappears entirely if the corresponding glomerulus becomes atrophic, while the remaining portions of the tubular system may persist.

In kidneys from both dogs and rabbits the afibrillar cell appears to be a smooth muscle cell which has become transformed into a cell of another type, without myofibrils, and with granules in the cytoplasm. The cellular transformation can be followed through by the observation of graded change from spindle cells with sparse granules to large epithelioid cells with numerous acidophilic and basophilic granules. Often the cells show mitotic figures or are multinuclear.

Recapitulated, the following changes [20–25] occur: (1) enlargement and lipoid infiltration of the cells of the juxtaglomerular apparatus, together with intercellular fibrosis; (2) transformation of smooth muscle cells to cells devoid of myofibrils, but presenting polychromic granules, vacuoles and hypertrophic nuclei; (3) vacuolization and intercellular lipoid infiltration of the intercalated segment; (4) atrophy of some glomeruli, enlargement of others and a decrease in their total number; (5) medial atrophy in renal arteries and arterioles; and (6) fibrotic patches in the interlobar arteries.

As I have indicated elsewhere,[18] afibrillar cells which contain polychromic granules intermingled with minute vacuoles are found in the superficial juxtaglomerular apparatuses of normal rabbits. The variations of their cytological features suggest the existence of a glandular cycle. The small granules which stain only after Zenker-formalin fixation are mitochondria. The large granules

are preserved by any fixative and are not a sign of retrogressive change, but may possibly indicate a stage in the formation of a vasopressor substance, perhaps an internal secretion of the afibrillar smooth muscle cell.

Discussion

Harrison and coworkers,[26] and Pickering and Prinzmetal [27] have reported extraction of a vasopressor substance from, and we have demonstrated the presence of granulated afibrillar cells in, the renal cortex of normal rabbits. In the normal animal the function of these cells may be that of the maintenance of vascular tonus. Since under the conditions of experimental renal ischemia these cells become larger and more numerous, it may be that the coincident hypertension is caused by their elaboration of a vasopressor substance.

In a study of the normal human kidney Becher [28] described cells which lie close to the arteriolar wall and ascribed to them an endocrine function. The term "Goormaghtigh-Becher cells," which Clara [12, 13] gave them, is inaccurate, as the cells which Becher observed are probably part of the intercalated segment, whereas our own observations have shown that the afibrillar granular cells occur only in the arteries and arterioles.

Although afibrillar cells are present in the arterioles of other organs, it is only in the kidney that they acquire granules, which is of significance when considered with the fact that ischemia of organs other than the kidney does not produce hypertension.[1, 29]

The proximity of the intercalated segment to the juxtaglomerular mass of afibrillar cells may imply some important functional relationship, and the observations described here demonstrate that pathological changes in one are always accompanied by changes in the other. That stimulation of the afibrillar cells is the exciting factor would seem probable from the following observations: Unilateral ureteral ligation in the rabbit does not lead to hypertension,[30] nor is there hyperplasia of the afibrillar cells in such animals. On the other hand, in scarlet fever, there is often hyperplasia and granulation of the afibrillar cells, and the transient hypertension may be followed by sustained elevation in blood pressure. Similarly, an adequate dosage of vitamin D_2 induces a marked hyperplasia of the afibrillar cells and these experimental

animals are hypertensive. Denervation of the carotid sinus and cardioaortic zones in dogs is followed by hypertension, and the kidneys exhibit hyperplasia of the juxtaglomerular apparatus, as Elaut has shown.[31, 32]

Summary

1. In dogs and rabbits renal ischemia is followed by hypertrophy and hyperplasia of afibrillar cells present chiefly in the walls of renal arteries and arterioles and in the juxtaglomerular apparatus. Accompanying this change there is atrophy of the ordinary smooth muscle cells of the arteriolar media and regression of some of the glomeruli.

2. In larger arteries afibrillar cells appear and lead to the subsequent formation of fibrotic patches in the arterial media and intima.

3. The close anatomical relationship which exists between the juxtaglomerular apparatus and the intercalated segment may indicate that cells of the segment in some manner influence the function of the afibrillar cells. Coincident pathological changes in the two structures are constantly seen.

4. Certain constantly observed anatomical features suggest that these specialized afibrillar cells secrete and liberate a pressor substance, and that in the hypertensive state this function is abnormally active.

Conclusions

On the basis of observations made by the author and others, it is suggested that a vasopressor substance may be formed in the afibrillar cells which exist in various portions of the renal vascular system, and that under certain conditions there may be liberation of an excessive amount of the pressor substance which leads to hypertension. The polychromic granules observed with appropriate fixation and staining may thus be actually incretory granules.

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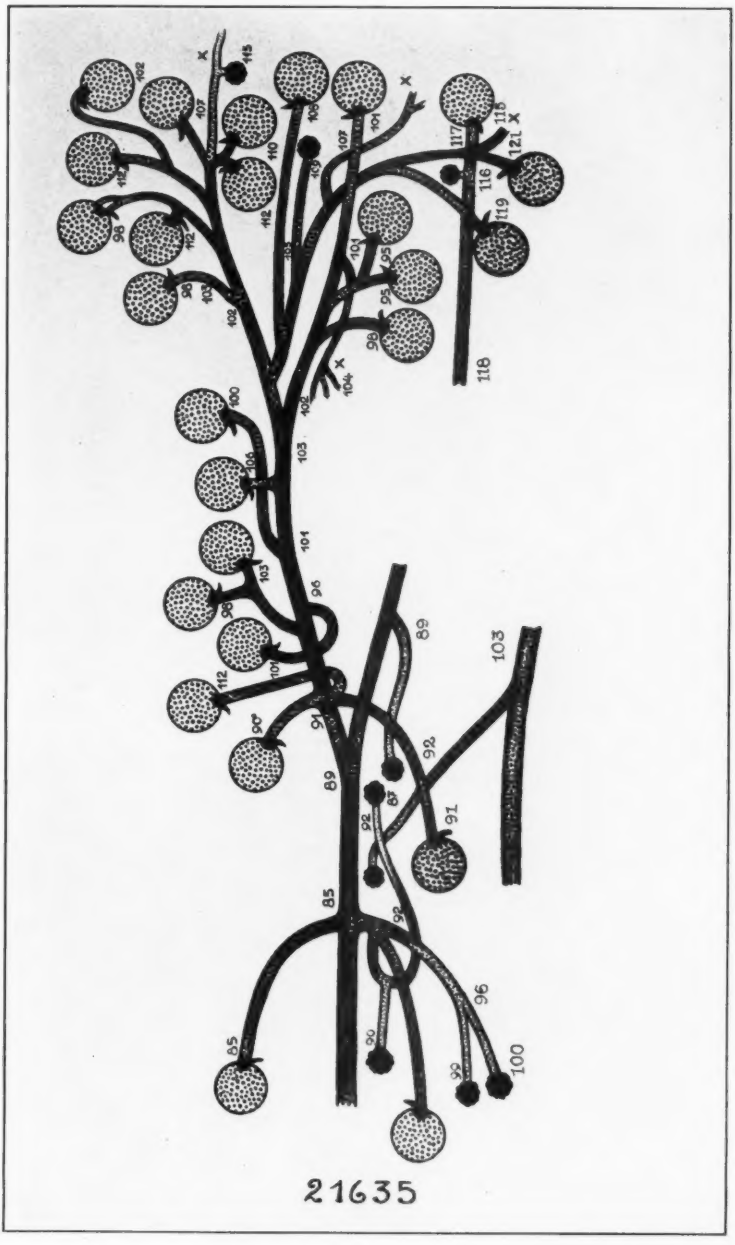
DESCRIPTION OF PLATES

PLATE 83

FIG. 1. Dog 21635. Renal ischemia 17 months. Diagram of a reconstructed interlobular prearteriole and its branches. The figures indicate the number of slides of a series (three sections on each slide). Enlarged glomeruli are stippled; atrophic glomeruli are solid black. At X are aglomerular Ludwig arterioles.



1

Goormaghtigh Histological Changes in the Ischemic Kidney

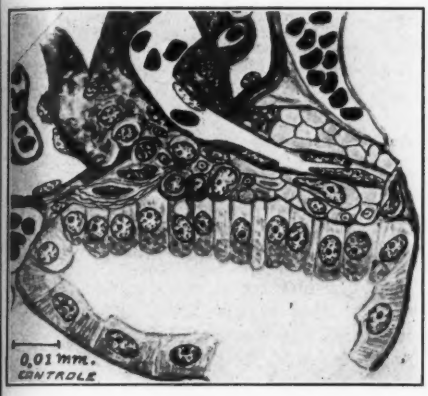
PLATE 84

FIG. 2. Normal dog. The vascular pole of a glomerulus. The afferent arteriole is cut in longitudinal section. The juxtaglomerular apparatus is composed mainly of afibrillar myoblasts with large nuclei. The columnar epithelial cells belonging to the adjacent intercalated segment are vacuolated at their bases. Zenker-formalin fixation. Masson's trichrome stain.

FIG. 3. Normal rabbit. The vascular pole of a glomerulus. The juxtaglomerular apparatus is composed of afibrillar myoblasts of varying size; some have clear cytoplasm, in others the cytoplasm is dark. In one large cell there are acidophilic granules. Bouin-Hollande's fixative. Masson's trichrome stain.

FIG. 4. Dog 24239. Renal ischemia 24 hours. The vascular pole of a glomerulus. The juxtaglomerular apparatus is enlarged and the afibrillar myoblasts are binuclear, vacuolated and swollen. There are vacuoles in the epithelium of the adjacent tubule. Bouin-Hollande's fixative. Masson's trichrome stain.

FIG. 5. Dog 24239. Renal ischemia 24 hours. An entire glomerulus. The juxtaglomerular apparatus is enlarged and afibrillar myoblasts bulge into the glomerular tuft. The vas efferens is seen in longitudinal section. There is vacuolization of the adjoining epithelium of the intercalated segment. Bouin-Hollande's fixative. Masson's trichrome stain.



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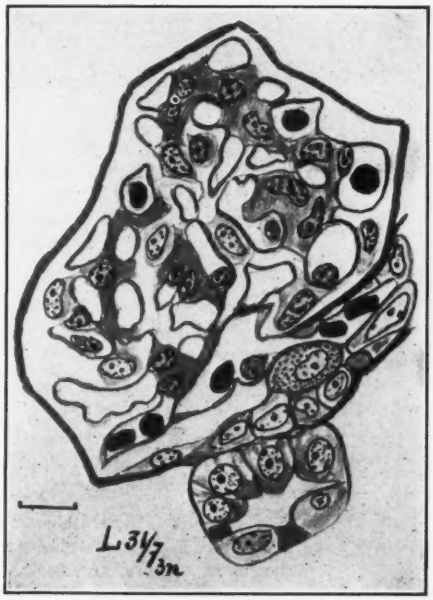

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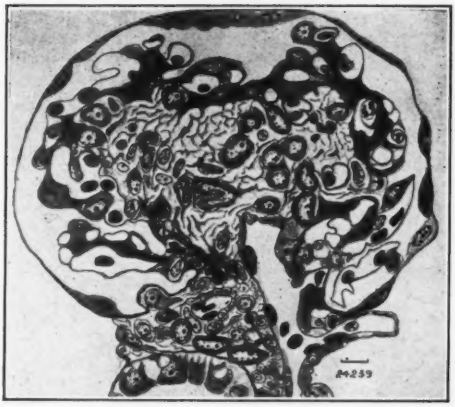


5

Goormaghtigh

Histological Changes in the Ischemic Kidney

PLATE 85

FIG. 6. Dog 21635 (26). Renal ischemia 17 months. The vascular channels are engorged. The glomerulus and juxtaglomerular apparatus are enlarged, and in the latter there are lipoid infiltration and discrete sclerosis. There are vacuoles in the epithelial cells of the adjoining intercalated segment, but the cells are of different structure than those not in contact with the juxtaglomerular apparatus. Zenker-formalin fixation. Masson's trichrome stain.

FIG. 7. Dog 21635. Renal ischemia 17 months. The distal end of an afferent arteriole. An afibrillar cell with granules is in contact with the vacuolated epithelium of the intercalated segment. Fixation and stain as in Figure 6.

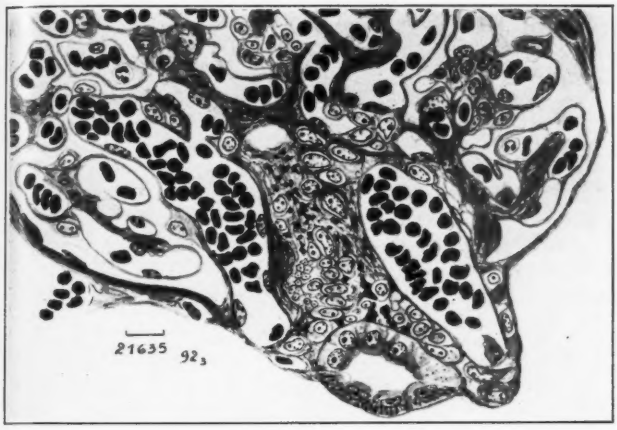
FIG. 8. Dog 30339. Renal ischemia 8 days. The bifurcation of an interlobular prearteriole. There are hypertrophy and hyperplasia of the afibrillar cells. Note the mitosis, the binuclear cell and the normal smooth muscle cells with distinct myofibrils. Bouin-Hollande's fixative. Masson's trichrome stain.

FIG. 9. Dog 21635. Renal ischemia 17 months. An interlobar artery. Notice the alteration in the medial smooth muscle cells and the thick intimal cushion of afibrillar myoblasts. Fixation and stain as in Figures 6 and 7.

FIG. 10. Dog 101138. Renal ischemia 8 months. A primary division of the renal artery. A multinuclear afibrillar myoblast lies in the midst of the atrophic medial smooth muscle cells. Bouin's fixative. Masson's trichrome stain.

FIG. 11. Dog 21635. Renal ischemia 17 months. The intercalated segment. A cell has become isolated from the segment which has undergone retrogressive changes. Zenker-formalin fixation. Masson's trichrome stain.

FIG. 12. Rabbit 31–96. Severe renal ischemia 3 weeks. A patent interlobular prearteriole. All of the smooth muscle cells have become afibrillar, and most of them contain polychromic granules. Notice that the nuclei are normal.



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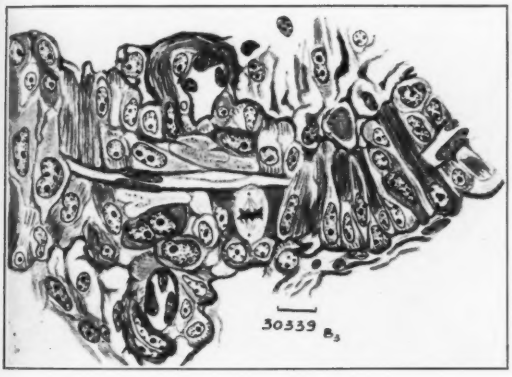

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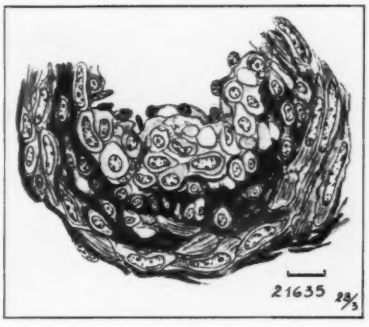

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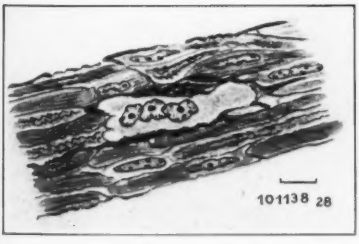

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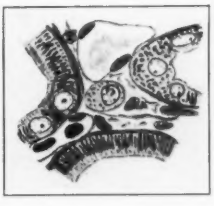

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Histological Changes in the Ischemic Kidney

HISTOGENESIS OF INDUCED PULMONARY TUMORS IN STRAIN A MICE *

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In 1896 during the course of some experiments on bacterial infections Livingood [1] discovered five spontaneous tumors in mice, one of which was a spontaneous lung tumor, apparently the first to be reported in the literature. This tumor was found in an albino mouse in which no other growths were noted and Livingood believed it to be an adenocarcinoma arising within a bronchus. During the period from 1905 to 1914 a large number of cases were reported and described in detail by Haaland,[2] Tyzzer,[3] Murray,[4] Jobling,[5] and Slye, Holmes and Wells.[6] All these tumors were believed to be spontaneous in origin and primary in the lung, although a number of them occurred in mice bearing tumors in other locations, particularly breast carcinomas. They were variously diagnosed as adenoma, papillary cystadenoma, or adenocarcinoma, and Tyzzer observed growths which he thought were epidermoid carcinomas. With the exception of this latter type, a reading of the descriptions and reference to the available illustrations strongly suggest the essential histological similarity of most of them. As to their point of origin, there was no absolute agreement among the various authors, although a great many were thought to arise from alveolar epithelium. Tyzzer [3] noted the resemblance of the tumor cells to bronchial epithelium, but on the other hand noted in one instance that the tumor was small and completely independent of the bronchi. By the use of serial sections Jobling [5] traced the origin of one growth to papillary outgrowths from the wall of a bronchiole. The largest series was studied by Slye, Holmes and Wells,[6] who reported their observations of 160 tumor nodules. They concluded that the tumors might arise from either the alveolar or the bronchial epithelium, but that it was extremely difficult to decide this point on the character of

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the tumor cells. However, they did believe that the papillary type of tumor arose more frequently from the alveolar epithelium.

Interest in these tumors apparently waned until 1925 when Murphy and Sturm [7] induced pulmonary tumors in mice by the repeated cutaneous application of a coal tar distillate. They gave no detailed report of the histological character of the tumors but illustrated a growth which is apparently identical with the type described by the earlier workers. Their work was soon repeated by a number of investigators, most of whom simply referred to the induced tumors as pulmonary adenomas or adenocarcinoma. With the establishment of the highly inbred "A" strain of albino mice by Strong [8] in 1921, a suitable test animal for the study of primary lung tumors became available. Bittner [9] has recently reported on the normal spontaneous incidence of lung tumors in this strain, and Andervont [10] has shown that practically all young mice of this strain will develop multiple lung tumors within 3 months following subcutaneous injection of suitable quantities of 1,2,5,6-dibenzanthracene.

We have had the opportunity in recent years of studying large numbers of these tumors in several strains of inbred mice, particularly the strain A from the Roscoe B. Jackson Laboratory. The induced tumors are almost invariably multiple, pearly white in color and situated close to the pleura so that most of them may be seen on gross inspection (Fig. 1). The tumors appear to grow expansively and are unencapsulated, being surrounded by compressed pulmonary tissue (Fig. 2). Microscopically most of them present a uniform picture of closely packed columns of cuboidal or columnar tumor cells arranged on a rather sparse stroma and with relatively few blood vessels (Fig. 3). The cytoplasm of the cells is generally smooth, slightly acidophilic, and the free borders of the cells are devoid of cilia. The nuclei are single, round or oval, and vary from vesicular to moderately deeply staining types. Mitotic figures, as in the spontaneous variety, are not numerous. Metastases seem to be infrequent although both lungs are frequently riddled with multiple growths.

It is obviously impossible when dealing with mature growths of this size to make any definite statement as to their site or mode of origin. However, with animals of known susceptibility, and carcinogenic hydrocarbons of relatively standard potency available,

we present in this paper our observations on the growth of the tumors during the first 3 months of their development.

Materials and Methods

In this experiment 200 strain A mice, obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, 2½ to 3 months old, and equally divided as to sex, were employed. 100 mice received 0.8 mg. of 1,2,5,6-dibenzanthracene in 0.8 cc. of lard subcutaneously; 60 mice received 1.6 mg. of methylcholanthrene in 0.4 cc. of lard subcutaneously; and 40 mice received 0.8 cc. of lard and served as controls. All injections were given at the same time and were made in both axillae and both groins in the hope of minimizing the development of subcutaneous tumors. However, animals that developed such growths, or died as a result of hemorrhage, infection or any other cause, were not included in the study. The final effective number consisted of 130 mice injected with hydrocarbons and 30 control animals. The mice were sacrificed daily except on Sundays and holidays, beginning on the day following injection, and the experiment was terminated in the 11th week. Since it was surmised that tumors would begin to appear about the 5th week, the greatest number of mice, a total of 98, were sacrificed between the 26th and 60th days.

In all cases the lungs were fixed by the intratracheal injection of not more than 0.5 cc. of Zenker's fluid plus 5 per cent of acetic acid immediately after the animals had been killed with illuminating gas. Tissues were embedded in paraffin and sections were cut at 6 to 7 μ. Complete serial sections of the right lower lobe were cut and stained routinely with eosin-methylene blue and with Goldner's [11] modification of the Masson trichrome stain. The remaining lobes were blocked in paraffin and served as reserve material. Some of this tissue was utilized for staining with Mallory's aniline blue for collagen, Foot's modification of the Bielschowsky method for reticulum, Giemsa's stain, and phosphotungstic acid hematoxylin.

Since it is not feasible to publish the complete descriptions of all the lungs examined, the observations are given on a fortnightly basis. It should be pointed out that the histological changes to be described were remarkably uniform among mice sacrificed at the

same time, and in many cases an individual protocol might be substituted for the combined summary.

Experimental Observations

1st and 2nd Weeks: Fifteen test animals and 4 controls were examined during this period. No gross lesions were observed and the lard was still present in the subcutaneous tissue at the sites of injection. Microscopically the alveoli were distended and empty, with the alveolar capillaries uniformly engorged with red cells. The bronchi were empty, moderately distended, and the epithelium cuboidal instead of columnar as a result of the intratracheal fixation. No changes could be seen in the bronchial epithelium aside from the slight mechanical flattening. Under higher magnifications there was seen a rather diffuse, though slight, cellular increase involving the alveolar walls, particularly in areas close to the pleura. The cells in question were approximately 15 to 20 μ in diameter by comparison with erythrocytes, and were mostly round or oval. They possessed a round, pale staining, central nucleus which occupied from one-third to one-half of the cell. There were usually one or two small prominent nucleoli and an occasional "one eyed" cell was noted. The cytoplasm was faintly acidophilic and very finely granular although no specific granules could be distinguished with the Giemsa technic. It was extremely difficult to ascertain the exact location of these cells in relation to the alveolar septum. None were noted free in the alveolar lumen. Some partly projected into the lumen from the septal wall, particularly in the angles formed by adjacent alveoli. They closely resembled the cells usually referred to as "alveolar phagocytes," although at this stage no phagocytosed material was seen within them nor was there any exudate or particulate matter within the alveoli which could be regarded as a likely object for phagocytic cells. In many instances these cells appeared to be within the alveolar walls although not within the capillary lumen, and none were observed within the lumens of arterioles or venules. There was no active proliferation as evidenced by mitotic activity or binucleate cells. Rather, they seemed to represent swollen cells of the alveolar walls. A few eosinophilic granulocytes were noted in the alveolar capillaries but none within the alveolar lumens. Perivascular and peribronchial lymphoid tissue, which is abundant

in these animals, appeared normal both quantitatively and qualitatively. No lesions were observed in the control material.

3rd and 4th Weeks: Twenty-four test mice and 6 controls were sacrificed. No tumors were found and the lungs grossly appeared normal. The lard was still present in most animals at the sites of injection in the form of thin walled oil cysts. Microscopically both bronchi and alveoli were well distended as a result of intratracheal fixation and were completely devoid of exudate. The bronchial mucosa was cuboidal and the cells appeared normal; there was no indication of any hyperplastic process. In most of the lungs, although by no means in all sections, there could be found localized collections of cells similar to those described above (Fig. 4). These focal accumulations were most noticeable close to, if not immediately beneath the pleura, and generally were more numerous and more cellular than noted in the first 2 weeks. As before, the shape of the cells varied considerably with their relation to the septal wall. Most of them were oval or rounded and were found for the most part in the angles between adjacent septal walls. The cytoplasm took the eosin stain rather faintly and was finely pebbled, but no specific granules could be seen in Giemsa preparations. Most of the nuclei were round and possessed a distinct nuclear membrane, and one or two small nucleoli which contrasted sharply with the clear nuclear background. A few cells could be seen lying free within the alveolar lumen and these were almost always rounded. There was still no exudate or foreign material to be found in the alveoli which would presumably elicit a phagocytic reaction on the part of alveolar lining cells. No mitotic figures were observed and there was no increase in the number of nucleated cells within alveolar capillaries although these were well filled with red blood cells. In 1 case there was a generalized hyperplasia of the peribronchial and perivascular lymphoid tissue and in many of these follicular collections there was definite increase in the number of eosinophilic granulocytes. No lesions were seen in the control material.

5th and 6th Weeks: During this period 38 test animals and 8 controls were sacrificed. Five of the test animals developed tumors during this period, the first being in a mouse injected with methylcholanthrene 32 days previously. The first tumor to be seen in a mouse receiving 1,2,5,6-dibenzanthracene was observed

36 days following injection. In the mouse sacrificed on the 32nd day only 1 tumor was seen, while in the others the numbers varied from 2 to 6, the latter being a mouse which had received 1,2,5,6-dibenzanthracene and which was killed on the 41st day. The tumors in all cases were located either in direct contact with the pleural surface or very closely adjacent to it, and all were minute, only 1 having been noted on gross inspection. In the animals that had developed multiple nodules there was considerable variation in size and some variation in their structure. The smallest, and presumably earliest lesions were composed of small columns or nests of cuboidal cells partially or completely lining the septums of several adjacent alveoli (Fig. 5). In many instances there was no distortion of pulmonary architecture, the alveolar lumens remaining patent but with their walls lined by cuboidal cells, thus producing a glandular appearance. The cells were rather uniformly cuboidal and possessed no cilia on their free borders. For the most part the cytoplasm was slightly acidophilic, although in moderate numbers it was definitely basophilic, and it almost always had an extremely fine granular or pebbled appearance. The nuclei were single, rounded and moderately large. The nuclear membranes were sharply outlined and in most cases there were one or two dark nucleoli set in a chromatin-poor nucleus. Smaller numbers had hyperchromatic nuclei and mitotic figures could be found only occasionally. In other instances the tumor nodules were composed of more closely packed cells which partially or completely filled the alveolar lumens (Fig. 6). However, there was no appreciable difference in the character of the individual cells.

No anatomical connection of these tumors with bronchi could be demonstrated in any instance and in most cases complete serial sections showed that the nodules were confined to a subpleural location considerably removed from the smallest bronchioles. However, there was a distinct resemblance between the cuboidal tumor cells and the lining cells of the bronchi which in these sections were cuboidal instead of the usual high columnar type seen in lungs which are not distended with fixative. As noted above, however, there were no cilia seen on the tumor cells.

Immediately surrounding practically every early tumor nodule were small collections of cells attached to the alveolar walls (Fig. 7). They occurred for the most part singly, although sometimes

small groups of two or three in a row might be seen. They varied in shape from spindled forms with both ends anchored to the septal wall to cuboidal types projecting into the lumen. In a few instances mitotic figures were observed in these cells. When occurring singly they could not be distinguished from the cells described in the protocols of the first 4 weeks, but when seen in small columns of two or three cuboidal elements they obviously were identical with the cells constituting the tumor nodules. The alveolar lumens were still free of fluid and cellular exudate. Alveolar capillaries were congested but no cells resembling tumor cells could be seen within them. Control material appeared normal.

7th and 8th Weeks: Thirty-two test animals and 7 controls were sacrificed during this period. Nine of the test mice had developed lung tumors, of which 7 were multiple. One test mouse also developed a lymphoma with multiple lesions in the spleen, lungs and lymph nodes. In most of the animals the lard solution was still to be found at the injection sites and in many of them there were areas of alopecia, and, in a few, small ulcers at the sites of injection. In 5 of the 9 mice with lung tumors the lesions were noted on gross inspection. It is probable that more tumors might have been seen grossly were it not for the fact that Zenker's solution obscures some of the smaller nodules. Microscopically the nodules varied considerably in size. Some were extremely small and were essentially similar to the type described during the previous period. Others were obviously larger and older. This would indicate that the lung tumors do not all spring up at the same time. Serial sections again demonstrated that in the case of the smaller and younger nodules they arose in the alveoli completely independent of the bronchial tree. The larger growths in general conformed to two types, although occasionally combinations of the two were noted. The more common consisted of closely packed, curving and sometimes sinuous columns of cuboidal cells. The intercellular boundaries were frequently prominent and gave the impression that the cells were forcibly pressed together. In general, the cells were identical with those described in the smaller nodules. However, there were greater numbers of larger cells with larger and darker nuclei; mitotic figures, while not numerous, were distinctly increased as compared with the smaller growths. These columns rested on a very thin core of connective tissue in which a small

capillary vessel could frequently be seen. With Foot's stain, the reticulum was unevenly distributed, in some areas appearing in rather coarse masses (Fig. 8), while in other parts it would occur in the form of very sparse delicate fibrils, or might even be completely absent.

In the second type of growth the alveolar structure could still be recognized (Fig. 9). Here the alveoli, in varying stages of collapse, were completely invested by a single or sometimes multiple layer of cuboidal tumor cells which did not differ in any respect from those in the more solid type. There did not seem to be any connective tissue save that of the alveolar walls, and the reticulum occurred only in the form of very fine, delicate threads, such as are seen in the normal alveolar wall. In some instances there was a combination of the two types, the solid growth usually being centrally located and the looser alveolar structure being found at the periphery (Fig. 10). In several instances these larger growths came in close contact with small bronchioles and in one instance at least completely surrounded a bronchiole. However, there did not seem to be any zone of transition between the two structures, the bronchial basement membrane remaining intact and the epithelial lining retaining its normal characteristics. In several instances during this period small clumps of phagocytosed pigment were seen within the cytoplasm of swollen alveolar cells, a few of which were lying free in the alveolar lumen. No changes of note were seen in control animals.

9th, 10th and 11th Weeks: Twenty-one test animals and 5 controls were sacrificed during this period and terminated the experiment. Eleven test animals had tumors in the right lower lobe and a single tumor nodule was found in a control animal. In almost every instance the tumors were seen grossly. Approximately half the test animals had developed ulcers at the injection sites and the remaining animals showed varying degrees of alopecia at the same spots. As noted previously, animals which had developed subcutaneous tumors were discarded from the study. Microscopically the tumors again varied in stage of development, although there were considerably fewer of the very small, early variety. In addition to the types described before there were several moderately large growths closely resembling the well

known mature type of adenomatous tumors (Fig. 11). These consisted of fairly solid papillary structures covered by a single layer of almost uniformly cuboidal tumor cells resting on a thin connective tissue core. Not infrequently a capillary or arteriole could be seen coursing through these nodules, although generally they were not abundantly supplied with blood vessels. In all the nodules the tumor cells were remarkably uniform and did not differ in any essential from the description previously given. Likewise in the alveoli immediately surrounding the tumor nodules there could be found the same swelling or actual proliferation of alveolar lining cells as noted before. In one instance a tumor nodule was found immediately adjacent to the open end of a terminal bronchiole and, as shown in Figure 12, it is almost impossible to distinguish the epithelial cells lining the bronchiole from the tumor cells. This may conceivably represent the origin of a tumor from bronchial epithelium, although the epithelium itself does not show any hyperplastic change. The resemblance between tumor cells and bronchial epithelium of course is accentuated by the flattened state of the bronchial lining. The solitary tumor occurring in the control animal was a small subpleural lesion in which the alveolar structure was still recognizable. This may represent a spontaneous tumor since pulmonary growths have been observed in untreated mice as early as 6 months of age.

Discussion

It is clear from an examination of this material that practically all these primary induced growths arose in the alveoli and not in the bronchi. The use of complete serial sections cannot be stressed too greatly for the fully developed tumor cells may closely resemble bronchial epithelium, particularly when it has become compressed to a cuboidal form by the intratracheal injection of fixing solutions. This point is extremely important when the tumors have come in more or less close contact with a bronchus in the course of their growth. The one tumor noted in intimate contact with a bronchiole may simply represent partial envelopment of the bronchiole by a tumor of alveolar origin. Livingood's [1] original paper described the growth as arising from within a bronchus, growing as a papilloma within it, and at one point breaking through its wall and dispersing in the surrounding tissue.

It is doubtful if this is convincing evidence of a bronchial origin, for we have frequently seen invasion of bronchi by larger tumors and Tyzzer [3] also observed this phenomenon in his series, suggesting that bronchial invasion might well be one route for intrapulmonary spread of the tumor.

Among recent workers who have studied the induced tumors Furth and Furth [12] believed that the origin of the tumors was from alveolar epithelium, although they felt that the relationship to bronchial epithelium was not clear. Campbell [13] has observed primary pulmonary tumors in mice exposed to dusts and tars of various types and is of the opinion that they usually originate from the alveolar cells as non-malignant tumors and then change more or less rapidly into a malignant type depending upon the degree of irritation. Magnus [14] regards these growths as papillomas arising from the bronchi with a strong tendency to malignant change.

The origin of these growths from alveolar lining cells is in sharp distinction to the origin of pulmonary carcinoma in man. Fried [15] particularly, as well as Geschickter and Denison,[16] Samson,[17] Frissell and Knox,[18] and many others, are almost unanimous in ascribing the origin of human pulmonary carcinoma to the bronchial epithelium. Cases of "alveolar carcinoma" in human beings have been reported especially during the period prior to Fried's monograph in 1932. However, recent workers are extremely sceptical of an alveolar origin and Geschickter and Denison [16] believe that its existence has never been fully demonstrated. In two other important respects the pulmonary tumors of mice differ from those of man. Practically all induced tumors and many of the spontaneous variety in mice are multicentric in origin, while the occurrence of primary multiple lung tumors in man is almost unknown. The location of the growths likewise differs greatly in the two species. Everyone who has examined the tumors of mice has been struck by their great predilection for the peripheral zones of the lung, frequently immediately beneath the pleura. In man, on the other hand, a great many and possibly the majority arise near the hilar region, although a peripheral form has been noted as arising from the terminal bronchi.

In 1925 Cowdry [19] described in detail a disease of South African sheep known as Jaagsiekte which in some respects closely resembles the lung tumors of mice. Jaagsiekte is believed to be an

infectious disease possibly of virus origin and characterized histologically by a remarkable proliferation of alveolar lining cells. In the fully developed form the alveolar walls are covered completely by cuboidal cells and the general structure is that of a papillary adenomatous growth. The cores of the papillae consist of the connective tissue of the alveolar walls. Cowdry's description of the histological evolution of the disease closely parallels the changes we have observed preceding and accompanying the development of lung tumors in mice. Similar lesions have also been observed by M'Fadyean,[20] Dungal [21] and others. Bonne [22] recently called attention to it and reviewed the literature, adding a case report of a Chinese who died with a diffuse, tumor-like lesion of the lung astonishingly similar to Jaagsiekte and which he describes as "carcinosis." However, this disease is a diffuse process and apparently is not characterized by the multicentric peripheral lesions seen in mice.

The most difficult point of interpretation in this study of course is the type of cell giving rise to the tumor. That it is usually derived from the alveoli is certain but the nature of the alveolar lining cells is far from certain. We do not propose to review the history of the controversy on the presence and nature of the alveolar epithelium. A full discussion may be found in the monograph of Miller [23] and in recent papers by Loosli [24] and Macklin.[25] It does seem clear that there are cells which line the alveoli; it is also probable that the lining is incomplete, for the evidence in favor of the normal existence of interalveolar communications is convincing. Miller and others believe that there is a true alveolar epithelium although it is difficult to demonstrate in normal lungs, and the lining cells seen in thick silvered sections are even more difficult to interpret. On the other side is Fried and many others who hold that there is no true alveolar epithelium but only mesenchymal cells which may or may not occur in sheets. The proponents of this view base their belief on the response of alveolar cells to the injection of particulate matter which is rather promptly ingested by phagocytic cells derived from the alveolar wall. There are grave doubts that phagocytic properties of themselves establish the non-epithelial character and mesenchymal origin of these cells. There does not seem to be any reasonable doubt that epithelium may under certain conditions exhibit phagocytic prop-

erties (Cowdry [26]). The crux of the problem rests obviously on embryological grounds, and Maximow and Bloom [27] have made this criticism. Here again there is a sharp cleavage of opinion. Most observers are agreed that the entodermal bronchial passages grow into a mass of mesenchyme which eventually gives rise to the blood vessels and connective tissue of the alveolar septums. Moreover, there is general agreement that the primitive alveoli are lined by cuboidal or columnar cells continuous with the bronchial epithelium. From this point on, however, there is no agreement. One school holds that the entodermal cells degenerate and disappear, leaving the underlying mesenchyme to line the alveoli. The other group believes that the entodermal epithelial cells do become greatly flattened but do not disappear, remaining as a true epithelial lining continuous with the bronchial lining. The issue is also taken into the field of tumor genesis, for Fried [15] believes that the mesenchymal lining cells of the alveoli are incapable of giving rise to an epithelial tumor.

Loosli [24] holds that the small nucleated cells which occur in the intercapillary spaces and which have been referred to under such terms as septal cell, pneumocyte, histiocyte, epicyte, macrophage, and pericyte are of mesenchymal origin and in inflammatory lesions of the lung are indistinguishable from the histiocytes of the perivascular and peribronchial connective tissue. Recently, Ross [28] has attempted to reconcile the conflicting evidence and suggests that there are actually two types of cell concerned and that they may be stimulated more or less selectively, depending upon the substances injected into the lung. The true alveolar epithelium is thus believed to be non-phagocytic, while the macrophage is phagocytic and capable of active migration across epithelial borders. Parker and Weiss [29] have shown that in patients suffering from advanced mitral stenosis there is a tendency for the flat epithelial cells to become cuboidal and their illustrations show a complete layer of such cells lining many alveoli.

Whatever may be the final solution of this perplexing problem, the development of induced adenomatous pulmonary tumors in mice offers a promising approach to the issue. The cells which were observed scattered in small foci close to the pleura preceding the development of tumor nodules, and also closely adjacent to the peripheral zones of expanding tumors, are indistinguishable

from alveolar phagocytes, and in a few instances contained small particles of pigment within their cytoplasm. In the earlier stages there did not seem to be any active proliferation of these cells, as shown by the rarity of mitotic figures, but the process seemed to be one of progressive swelling and enlargement. At slightly later stages, however, mitotic division was noted both in the alveolar cells adjacent to the tumor nodules and in the tumor cells themselves. The result of this steady enlargement and proliferation was a more or less complete investment of alveolar walls by a layer of cuboidal cells which morphologically appeared to be epithelial. That a similar lesion occurs in the development of spontaneous tumors is strongly suggested by an observation recorded by Tyzzer.[30] In 1 case in his first paper he noted that in addition to definite tumor nodules there were masses of cells occasionally in mitosis filling the air spaces and closely resembling swollen cells of alveolar epithelium. There was slight evidence of phagocytic activity on the part of these cells. Tyzzer was uncertain whether this lesion represented a diffuse form of tumor or simply a reaction on the part of alveolar epithelium to the presence of definite tumors. With this interpretation of tumor development the alveolar lining cell, whatever its embryological origin, might be capable of differentiating first into a cell indistinguishable from the alveolar phagocyte and then into an epithelial form. However, this hypothesis cannot be accepted without further detailed studies of the phagocytic properties of the tumor cells and their behavior on transplantation. The connective tissue framework in the early stages of the pulmonary tumors studied in this paper appears to be furnished by the septal wall. It is rather surprising that so little mention is made by histologists of this form of tumor in discussing the nature of the alveolar lining cell, although Cowdry [26] makes a passing reference to the occurrence of a lung tumor derived from alveolar epithelium.

The absence of any recognizable inflammatory reaction in the lungs preceding tumor development should be noted. Studies by numerous investigators suggest that the presence or absence of inflammatory reactions, preceding or accompanying tumor formation, probably depends on several factors, including the mode of administration of carcinogen, as well as the concentration and character of the solvent or vehicle. It seems highly improbable

that inflammatory reactions are a necessary accompaniment of the histogenesis of tumors.

Conclusions

Pulmonary tumors induced in strain A mice by the subcutaneous injection of 1,2,5,6-dibenzanthracene or methylcholanthrene begin to appear 5 weeks after injection.

Practically all of these growths are of alveolar origin and are not associated with an inflammatory reaction.

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DESCRIPTION OF PLATES

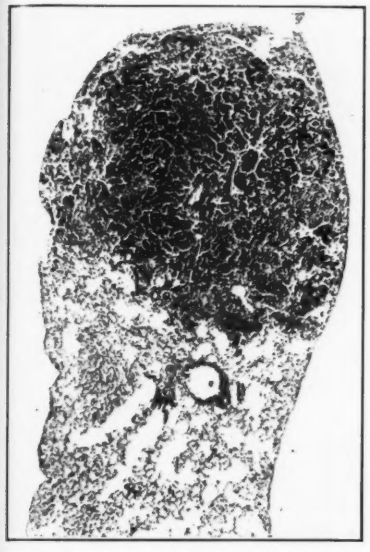
PLATE 86

FIG. 1. Dorsal surface of the lungs of a strain A mouse showing multiple primary tumors on the surface. × 3.8.

FIG. 2. A pulmonary tumor of moderate size illustrating the common location and lack of encapsulation. Hematoxylin and eosin stain. × 48.

FIG. 3. A fully developed pulmonary tumor composed of cuboidal and columnar cells in papillary glandular arrangement with little stroma. Note also the paucity of blood vessels. Hematoxylin and eosin stain. × 200.

FIG. 4. A localized subpleural collection of alveolar cells of varying shape. Several appear to be rounded and free in the alveolar lumen. Eosin-methylene blue stain. × 680.



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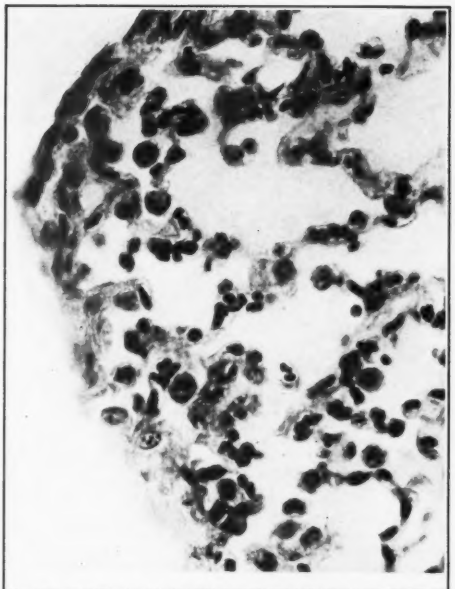

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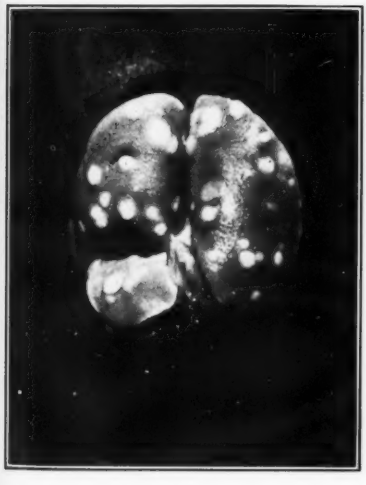

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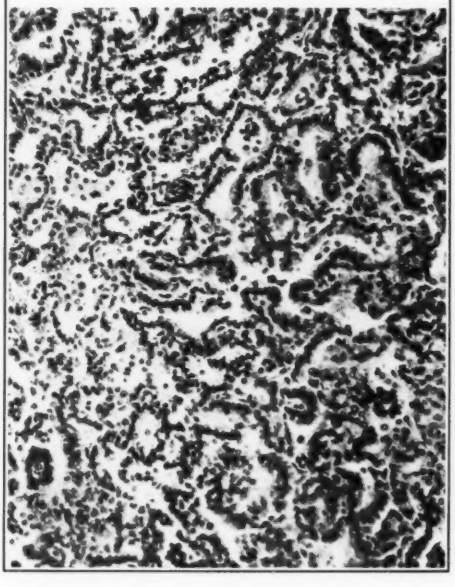


3

Grady and Stewart

Induced Pulmonary Tumors in Strain A Mice

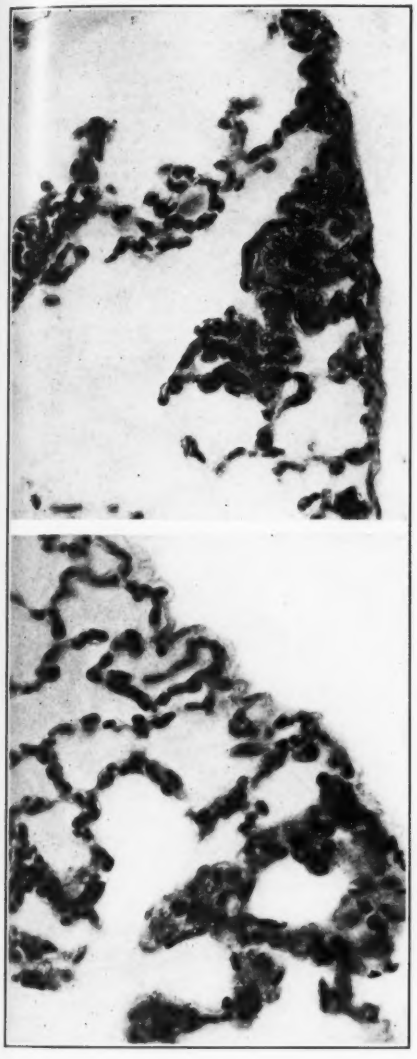
PLATE 87

FIG. 5. An early growth involving several adjacent alveoli. The alveolar pattern is intact and the alveolar capillaries are still visible. Eosin-methylene blue stain. × 680.

FIG. 6. A more solid type of early growth with tumor cells filling up the alveolar lumen. Eosin-methylene blue stain. × 680.

FIG. 7. The edge of an early growth is seen in the lower portion of the field. In the center is a single alveolar cell in mitosis and in the lower left corner is a group of three tumor-like cells arising from the alveolar wall. Eosin-methylene blue stain. × 680.

FIG. 8. The reticulum is distributed in coarse strands about groups of tumor cells. Foot's modification of Bielschowsky's method. × 680.



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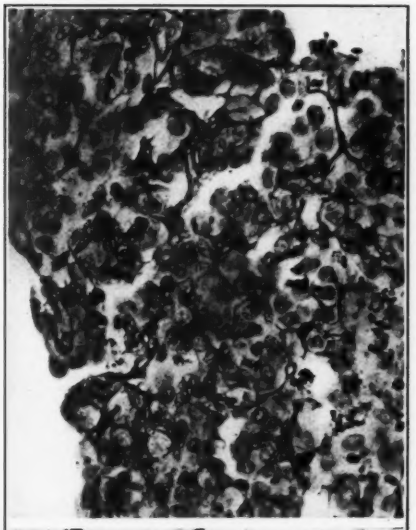

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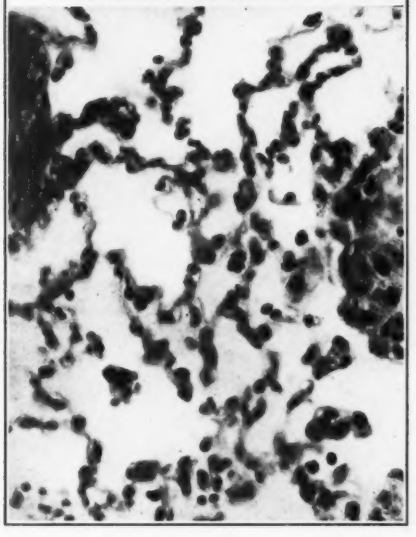

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Induced Pulmonary Tumors in Strain A Mice

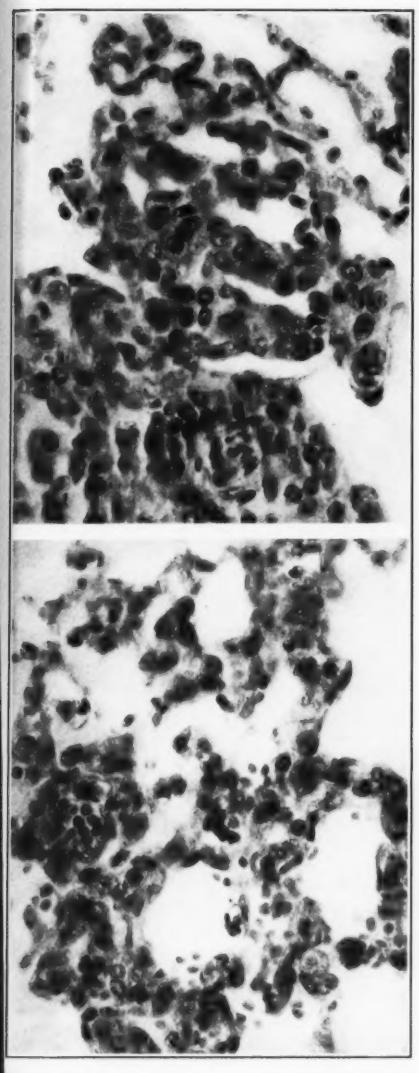
PLATE 88

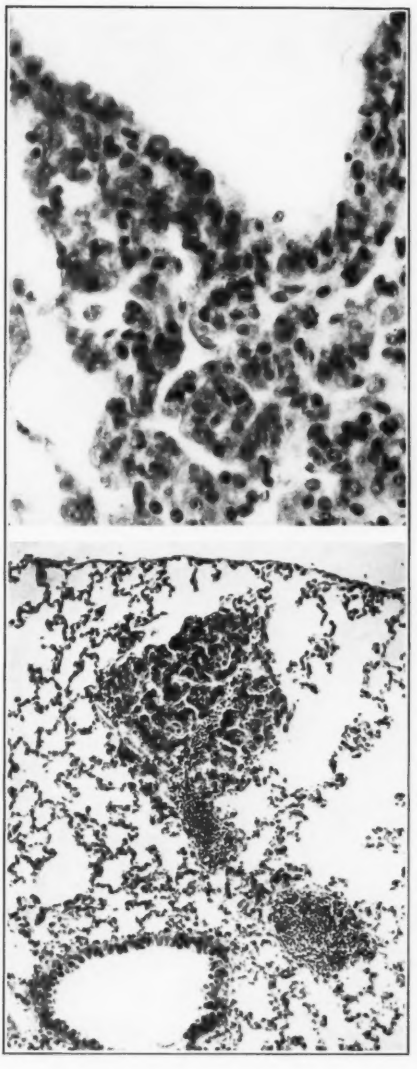
FIG. 9. The alveolar structure is still apparent although the walls are almost completely invested by tumor-like cells. Eosin-methylene blue stain. × 680.

FIG. 10. The peripheral portion of a moderately large growth has a loose alveolar structure. The central, more solid portion is only partially visible at the left. Eosin-methylene blue stain. × 680.

FIG. 11. A typical adenomatous tumor close to the pleura is almost completely bisected by a small blood vessel. Eosin-methylene blue stain. × 200.

FIG. 12. A tumor in intimate relationship with a bronchiole. The bronchiolar lining to the right is cuboidal and closely resembles the tumor cells. Eosin-methylene blue stain. × 680.





Grady and Stewart

Induced Pulmonary Tumors in Strain A Mice

THE HISTOLOGICAL LESION IN LYMPH NODES IN INFECTIOUS MONONUCLEOSIS *

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Since infectious mononucleosis is a self-limited non-fatal disease, the diagnosis of which is readily accomplished by hematological and serological means, there has been little recourse to diagnostic biopsy. As a result, comparatively few studies have been made of lymph nodes removed during the active stages of the disease and from these no constant specific changes have been recorded.

The importance of determining the characteristic anatomical features of this lesion may be appreciated by referring to the comments of various competent observers who have noted the similarity of the structure of lymph nodes in glandular fever to certain malignant diseases of lymphoid tissue. It is worthy of note that since these observations were made almost two decades have elapsed, and it is probable that opinions would be less equivocal at this time. Sprunt and Evans in 1920 [1] reported the histological studies of nodes from 3 cases of glandular fever. One node was examined by Welch and Whipple who believed that no definite diagnosis could be made but stated that lymphatic leukemia could not be ruled out. A node from the 2nd case, examined by Martzloff, was said to show lymphadenitis, although Bloodgood suggested that it might be "the earliest case of Hodgkin's disease ever studied." A node from the 3rd case was described by MacCallum, who stated that it represented a picture consistent with lymphatic leukemia, and if it were that, it was either in a very early stage or in a period of recovery. Bloodgood, who reviewed the same section, said that no definite diagnosis could be made but that lymphosarcoma could not be excluded. Longcope [2] (1922) described the nodes from 2 cases and said that both resembled Hodgkin's disease but that neither was sufficiently characteristic to permit that diagnosis. Nelken [3] (1 case) in 1926 observed that

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the entire picture was that of diffuse hyperplasia suggesting early lymphatic leukemia. Although more recent authors have not stressed the question of similarity to malignant disease, it is obvious that these earlier observations lend a degree of importance to a more definitive analysis of the lymph node lesions occurring in this disease. Occasionally, even with current refined diagnostic methods, a clinical diagnosis of infectious mononucleosis is missed either through oversight or because the disease has run an unfamiliar course. Under such circumstances a diagnostic biopsy may, and probably has, reached the pathologist. The need for the appreciation of the specific lesion underlying the disease therefore becomes evident.

Review of the Literature

Before proceeding to an analysis of our series it is considered of significant interest to summarize the observations of other authors, many of which have served to corroborate and facilitate interpretations of our own. We have arranged this summary according to the general characteristics and the appearance of the various anatomical constituents of the lymph node (*i.e.*, sinuses, follicles, lymph cords and component cells) in order to separate the general problem into its structural subdivisions.

General Features: Downey and Stasney,[4] whose review of this subject and detailed study of six lymph nodes is unusually comprehensive, noted that nodal architecture was irregularly preserved and one part of a node was found to be normal while other parts were hyperplastic and the normal structure obliterated. Hyperplastic phenomena were not limited to the lymphoid elements but involved as well the sinus endothelium and reticulum cells. Inconstant hyperplasia was also observed by Sprunt and Evans (3 cases). Glanzmann[5] (number of cases not recorded) stated that the picture was non-specific, and that of a hyperplastic lymphadenitis. Hartwich[6] (1 case) remarked that the node was richly cellular and that the follicles blended indistinguishably with the pulp. Baldridge, Rohner and Hansmann[7] (6 cases) described marked hyperplasia with expansion of the capsule. Nelken (1 case) noted a "washed out" structure followed later by a return to normal architecture. Pratt[8] (1 case) found active

hyperemia, a serous exudate, and early degenerative changes in blood vessel walls. Fox [9] (2 cases; 1 lymph node and 1 tonsil) and Chevallier [10] (number of cases not stated) recorded both lymphoid and reticulum hyperplasia.

Sinuses: The sinuses were obliterated in some of the cases described by Downey and Stasney but were present in other instances and showed hypertrophic reticulum cells. Advanced cases showed sinuses filled with reticulum cells, an observation concurred in by Nelken, Hartwich, Longcope, and Sprunt and Evans. Glanzmann found marked proliferation of sinus endothelium, a condition termed by him "sinus catarrh." Baldridge, Rohner and Hansmann observed the sinuses to be compressed. They found mitoses in the wall of the sinuses and lymphoid cells free in the lumens. Marchal, Bargeton and Mahoudeau [11] (1 case) recorded the presence of a dilated marginal sinus with endothelial hyperplasia. A preponderance of small lymphocytes and a moderate number of phagocytes were seen in the sinuses by Fox. Chevallier found large macrophages resembling giant cells with one or more nuclei and also swollen endothelial cells. Sprunt and Evans in one instance observed the peripheral sinuses to be plainly visible but those in the central portion of the node were obliterated.

Follicles: No follicles were seen in 1 of the cases of Downey and Stasney but they were absent only in the hyperplastic areas of another. In a 3rd, follicles could be recognized but there were no germinal centers. In advanced cases no follicle or germinal center tissue was seen at all. Failure of consistent follicular arrangement was also noted by Sprunt and Evans and by Fox. Glanzmann [5] and Vogl [12] (1 case) found that follicular cellularity was diminished, but on the contrary Baldridge, Rohner and Hansmann, Hartwich, and Longcope described germinal center hyperplasia associated with many mitotic figures. Marchal, Bargeton and Mahoudeau described follicular hyperplasia with increased germinal center elements. There was also hyperplasia of reticulum cells at the borders of the follicles. Nelken found that follicles were not prominent early in the disease but later in its course they were, and there was hyperactivity of germinal centers. Wiseman [13] (2 cases) found germinal center tissue infiltrating the entire lymph node early in the disease but this became less obvious later, with uniform admixture of cells.

Lymphoid Cords: Nodes studied by Downey and Stasney showed crowding of small lymphocytes in the cords and among these cells there were larger, more loosely arranged lymphocytes. In addition, there were scattered nodular clusters of swollen rounded reticulum cells producing a characteristic "spotty appearance." After the peak of the disease all of the nodes showed nodular reticulum cell hyperplasia in all areas except the region of the follicles. Similar multiple nodular areas have been noted by Pratt and by Marchal, Bargeton and Mahoudeau. Pratt observed central necrosis in some of these nodules and a node removed a year later showed nodular fibrosis. Wiseman found germinal center tissue consisting for the most part of "prelymphoblastic" elements infiltrating the lymphoid pulp early in the disease. Longcope noted that a few of the large cells found in the sinuses infiltrated among the lymphocytic elements of the cords.

Cellular Characteristics: Downey and Stasney stated that the great majority of the cells in the nodes were small lymphocytes, normal in appearance except for a densely basophilic cytoplasm and an unusually coarse chromatin. There were gradations between these cells and large lymphocytes with mature nuclei but deeply basophilic cytoplasm. Some of these exhibited transitional stages leading to the formation of plasma cells and there were also still larger elements with indented or even lobulated nuclei. Transformation of reticulum cells to lymphocytes was also described. The cells in Baldridge, Rohner and Hansmann's cases varied markedly in size and showed irregularly lobulated nuclei. Dwarf and pathological lymphocytes with polymorphic nuclei, atypical plasma cells, lymphoblasts, and peculiar monocyte-like elements were described by Nelken. Marchal, Bargeton and Mahoudeau stated that lymphocytes predominated but there were many monocytes in the loose reticulum meshwork and masses of "tumefied" elongated histiocytes were seen which occasionally simulated new blood vessel formation. Fox, who stated that 95 per cent of the cells were lymphocytes, also observed large plasma cells and large elements with eosinophilic cytoplasm and irregularly shaped, well stained nuclei containing one or two clear nucleoli. Similar cells in the sinusoids had neutrophilic cytoplasm and exhibited phagocytosis of erythrocytes. In one node Sprunt and Evans described many lymphoid and endothelial cells, and in another many large

cells with pale staining, round or oval nuclei, many neutrophils and monocytes.

Although most of the aforementioned authors have made clear-cut analyses of their own material, a combined analysis of all the various observations published produces a considerable degree of confusion. This is to a great extent the result of the pernicious variation in terminology which has dogged the study of hematology since its inception. Of equal significance is the fact that an insufficient number of biopsies have been available for study by individual observers. As a result changes which have represented a single phase of the disease have been taken by some to represent the fundamental lesion. It is our belief that this situation will be clarified by the study of the cases in our series in which biopsies were made at various stages of the disease.

Material and Technic

Specimens adequate for histological study have been obtained by biopsy of enlarged lymph nodes from 10 cases during the active phases of infectious mononucleosis. The nodes were sectioned immediately and almost all were fixed in Zenker's fluid plus acetic acid. The tissue was embedded in paraffin, sectioned, and stained with phloxine-methylene blue. On occasion, portions of the material were used for imprint preparations, which were subsequently stained with Wright and Giemsa stains, and several times small amounts were aspirated from the freshly cut node and utilized for supravital studies after staining with neutral red and Janus green. Some of the material was fixed in formalin and utilized for special reticulum stains. On the Zenker-fixed specimens additional studies were made by means of the Mallory aniline blue and phosphotungstic acid hematoxylin stains.

Table I summarizes the sex, age and occupational status of each patient from whom biopsy material was obtained. There is further tabulated the day of the disease on which the biopsy was made, the character of the heterophile antibody reaction, the height of the red and white blood cell counts, and the percentage of mononuclear cells in the peripheral blood at the time the biopsy was done. All mononuclear cells are grouped together in this instance because of the varied and inconclusive terminology (mononuclear cell, monocyte, lymphocyte, large mononuclear) used by the dif-

TABLE I

Clinical Data

Case number	Sex	Age	Day of disease	Occupation	Heterophile antibody Titer	Red blood cell count (million)	White blood cell count (thousand)	Mononuclear cells
		yrs.						%
1	F	25	8	Housewife	1:128	4.7	12.0	49
2	M	28	28	Laborer	Not done	Not done	14.0	75
3	M	28	28	Businessman	1:128	5.7	12.5	69
4	M	6	21	Student	1:128	4.7	18.0	83
5	M	19	16	Student	1:128	3.7	9.5	74
6	F	28	14	Student	Positive; titer not given	4.7	16.0	75
7	M	24	5	Physician	1:128	4.8	12.0	50
8	M	16	18	Student	1:128	4.9	14.5	77
9	F	12	8	Student	1:16	Not done	17.8	64
10	M	27	14	Student	1:128	Not done	16.6	74

ferent examiners. The determination of the heterophile agglutinin titer was not done in Case 2 and was only weakly positive in Case 9. The determination in this case was done early in the disease and was not repeated at a later date. It is altogether possible that it may have become more strongly positive later, since all of the other features of the syndrome developed in a characteristic fashion.

It is apparent that all cases were biopsied during the active stages of their illnesses, as evidenced by the blood pictures recorded. It is further obvious that an excellent opportunity is afforded for tracing the pathogenesis of the lesion since the nodes were removed at varying intervals from 4 to 28 days after the onset of symptoms.

Definition of Terms

In order to avoid further conflict in nomenclature with that which already exists, it is deemed advisable to define the histological phraseology which we have adopted before proceeding with the analysis of our cases. Our conception of the anatomy of the normal lymph node is in keeping with that expressed in commonly used textbooks of histology.[14, 15] Certain terms, however, require more definitive description.

Reticulum: Emerging from the capsulotrabecular system, optimally visible with special stains only, is a delicate fibrillar network of argentophilic material which is termed reticulum. The meshwork is particularly loose immediately adjacent to the capsule and trabeculae, in which regions may be found the marginal and radial sinuses respectively. These sinuses are traversed by reticulum fibrils which become condensed to form the sinus walls and proceed therefrom internally to form a netlike framework for the remainder of the node.

Germinal Centers: In the center of the follicles, cells are loosely aggregated and consist of various types. In the minimally active follicle there are small lymphocytes and a few less mature, somewhat larger elements termed lymphoblasts. In the hyperactive or hyperplastic follicles less mature cells predominate and there are large numbers of lymphoblasts and masses of cells with large vesicular nuclei containing scanty chromatin and prominent nucleoli. These elements possess abundant basophilic or amphophilic

cytoplasm which is poorly delimited and fused with that of adjacent cells. These have been termed reticulum cells by some, but since it is our belief that they are undifferentiated hematopoietic precursors and have little to do with the production of reticulum, we have preferred the name "stem cells" (Fig. 5).

Sinus Cells: Within the lumens of the sinuses, either attached to the mural or intrasinus reticulum, or lying free in the interstices, are cells measuring 15 to 20 μ in diameter which show relatively large, somewhat lobulated or horseshoe shaped nuclei with abundant eosinophilic, phagocytic cytoplasm. These have also been called reticulum cells by some and by others reticuloendothelial, endothelial, or even resting wandering cells (Fig. 5). Since they have nothing to do with the formation of reticulum and are morphologically indistinguishable from cells which are known as histiocytes or clasmatocytes when encountered elsewhere in the body, we have adopted the latter name for them.

Observations

The basic lesion of infectious mononucleosis is apparently the result of the varied responses of several different elements composing the lymph node to a single, presumably irritative stimulus. The structures manifesting this reaction may be enumerated as follows: (*a*) lymphoid follicles; (*b*) lymphoid cords; (*c*) lymph sinuses; (*d*) sustentative elements and blood vessels.

The reactions of many of these are essentially banal and of no specific diagnostic value individually. In combination with each other, however, a histological picture is produced which is characteristic and fundamentally unlike any described for those conditions which may clinically mimic the disease (*i.e.*, malignant lymphoma, lymphatic leukemia, systemic glandular tuberculosis, and so on).

The important crude diagnostic feature which serves in the differentiation from primary neoplastic disease of the lymph node is the retention of gross architectural relationships. This is particularly the case with reference to the persistence of subcapsular and radial sinuses. In this connection, therefore, it is important that a word of caution be appended regarding the fixative to be utilized in the preparation of biopsied lymph nodes. In nodes

prepared in part by fixation in formalin or Bouin's solution and in part by fixation in Zenker's fluid with acetic acid, subsequent embedding in paraffin and sectioning has revealed a rather striking variation in appearance. The shrinkage of the formalin or Bouin-fixed paraffin sections has been sufficiently great so that in combination with the distortion already produced as the result of the disease process itself, the appearance of total architectural obliteration is simulated. This confusing feature is readily avoided by the less apparent shrinkage in the Zenker-fixed tissue. Furthermore, in view of the relatively specific staining qualities imparted by phloxine-methylene blue to the so-called infectious mononucleosis cell, it is recommended that biopsied lymph nodes in cases of suspected infectious mononucleosis always be fixed in Zenker's fluid and stained with these dyes. The descriptions herein recorded are based almost wholly upon lesions studied in nodes treated in this fashion.

Since the disease may exist for a variable period before clinical manifestations appear, it is not possible to establish accurate time relationships with any degree of assurance. Any attempt to do so must be considered pure conjecture.

The basic process underlying the lymph node lesion in this disease is essentially the result of proliferative stimulation of the components of the node. It is probable that sustentative and vascular elements are only secondarily affected but since all tissues respond almost simultaneously, it is not valid to make this an unequivocal premise.

In what appears to be the early stages of the lesion the germinal centers of the lymph follicles become hyperplastic and show large secondary nodules (Fig. 4). These consist of masses of apparently fused cells with abundant, poorly defined, basophilic cytoplasm and large vesicular nuclei (stem cells), and also of varied numbers of mononuclear elements with more sharply defined and demarcated eosinophilic cytoplasm and eccentrically placed lobulated or reniform nuclei (probably clasmatocytes). Mitotic figures are numerous and there is evidence of phagocytic propensity among many of the clasmatocytes.

At the same time there are increased numbers of mitotic figures in the larger cells of the extrafollicular lymphoid substance in both cortical and medullary regions. Whereas the follicular

changes noted above are entirely non-specific, significant numbers of mitotic figures in the pulp are distinctly unusual in the ordinary type of hyperplasia. The majority of the cells in these regions are small and normal appearing lymphocytes with collar-like packing at the periphery of the follicles. An interadmixture with increasing numbers of larger cells becomes rapidly apparent. These are approximately two to three times the size of the small lymphocytes and possess abundant basophilic, either granular or relatively coarsely vacuolated, sharply delimited cytoplasm (Fig. 5). Phloxine and methylene blue impart a filmy blue quality to the cytoplasm which simulates the appearance of mucin. This staining characteristic is in distinct contrast to the blue and dark purple cytoplasm noted in the lymphocytes and stem cells respectively. We have never noted such cells as these in significant numbers in lymph nodes in other clinical conditions. Nuclei are vesicular, round or slightly indented, and eccentric in position. Imprint preparations from the fresh lymph node stained with Wright and Giemsa stains demonstrated these specific cells to be identical with the large mononuclear elements in the blood stream believed to be peculiar to infectious mononucleosis. Although these have been mistakenly described as monocytes, supravital studies have shown them to be unquestionably large atypical lymphocytes.[16] For purposes of simplicity these will be termed infectious mononucleosis or I.M. cells throughout the remainder of this presentation. Mitotic figures are particularly numerous among these elements in the cords although several of the cells exhibit evidence of amitotic division. In addition to I.M. cells and small lymphocytes there are also increased numbers of stem cells and lymphoblasts scattered throughout the pulp.

Associated with the proliferation of the pulp cells there is an apparent increase in the amount of fibrillar supportive reticulum per unit area. This may, however, be more apparent than real and simply be the result of peripheral compaction of reticulum by the pronounced expansion of hyperplastic follicles. This is perhaps the case also in the seeming increase in the number of stromal blood vessels. In these, however, there is definite evidence of intrinsic proliferation exemplified by increased numbers of endothelial lining cells, some of which exhibit mitotic figures. The endothelial elements become swollen and assume an epithelial-

like appearance and as a result occasional vascular channels simulate the appearance of glandular acini.

With continued cellular proliferation the subcapsular and radial sinuses show progressive compression and distortion. They are not, however, ever completely obliterated, a feature which should be determined on the basis of Zenker-fixed tissue for the reasons given above. Lining elements of the lymphoid sinuses also show evidence of proliferative stimulation (Fig. 3). These phagocytic cells or clasmatocytes become swollen, their nuclei vesicular, but the cytoplasm retains its eosinophilic hue. As the result of fairly rapid division they tend to form small masses or islets at the edge of the sinuses. The sessile islets project into the sinus lumens and peripheral cells are freed and lie in fairly significant numbers as discrete elements. Phagocytic activity is demonstrated by cytoplasmic vacuolization and engulfment of cellular substance and detritus. The staining qualities of the cells in fresh imprint preparations and supravital spreads are those of monocytes and related elements.

Sinuses having been markedly obtruded upon by the pronounced increase of parenchymatous substance are narrow and irregular but definitely retain their identity. In addition to the phagocytic cells described above there are also varying numbers of lymphocytes and I.M. cells lying within the sinuses (Fig. 2). The latter named cells appear as large (15 to 25 μ) mononuclears with abundant basophilic and fairly granular, frequently mucin-like cytoplasm. Nuclei are eccentric, slightly indented or lobulated, vesicular, and contain a fine reticulated chromatin with nucleolar condensations. Mitotic figures and even double nuclei are not unusual in these cells. With progression of the lesion the free lying cells increase in number in both peripheral and medullary sinuses and often appear as loosely compacted masses not unlike similar masses observed in the early phases of lymphatic leukemia (Fig. 1). In addition they are also apparent among the fibrous components of both the nodal capsule and the trabeculae. There is never, however, sufficient accumulation or activity in these regions to simulate neoplastic invasion. Nor do they ever appear in significant bulk in tissues beyond the nodal capsule.

The size of the lymphoid follicles is remarkably variable but they tend to enlarge throughout the course of the process (Figs.

4 and 6). Germinal centers, hyperactive throughout, produce in many gradual narrowing of the surrounding collar of small lymphocytes. In such instances this layer becomes less compact and shows a scant intermingling with stem and I.M. cells (Fig. 6). During the latter phases of the disease follicular hyperplasia may progress to such an extent that spherical configuration is no longer retained. The apparent follicles, now consisting for the most part of hyperplastic germinal centers, are irregular in shape and exhibit broad pseudopod-like extensions into the surrounding pulp. There is progressive fusion and commingling of follicular and pulp content (Fig. 7). Ultimately the lymphocytic collars disappear entirely and although a shadow-like remnant of concentrically arranged reticulum may be noted with silver stains, the lymphoid cords become broad heterogeneous masses of syncytial stem cells, mature small and large lymphocytes, and I.M. cells, among which are numerous mitotic figures.

The simultaneous proliferation of sinus wall phagocytic elements at this stage proceeds in a centripetal as well as a centrifugal fashion. As a result small clusters of pink staining mononuclear phagocytes become evident within the lymphoid pulp immediately adjacent to the sinus walls (Fig. 3). These rapidly increase in size and contrast with the surrounding basophilic cells so that with the low power objective the moth-eaten appearance described by Downey and Stasney is evident. Tangential sections frequently show what appear to be isolated islets of these eosinophilic elements apparently separated from their sinus attachment in the midst of the hyperplastic cords (Fig. 8). Indeed, progressive proliferation permits further extension into the lymphoid pulp and there is admixture of the active macrophages and monocytes with an already polycellular pulp. Focal aggregations of these eosinophilic cells suggest but have only a bare resemblance to tubercles.

During the florid stage of the disease the appearance of the lymph node is quite unusual and presumably specific. The node is enlarged and shows greatly increased cellularity in the medullary, cortical and sinus substance. The cords are swollen by a rich mixture of small and large lymphocytes, stem cells and lymphoblasts, I.M. cells, and large eosinophilic phagocytic elements (Fig. 7). Follicles persist in some but in others are apparent only

as occasional, partially disrupted germinal center fragments, and reticulum stains exhibit a vestige of concentric perifollicular arrangement. There is marked sinus compression and distortion although identity is preserved. The sinus lumens contain variable numbers of cells similar to those noted in the pulp. Cells of this type are also evident in small numbers in both the trabeculae and capsule of the node. There is an apparent increase in reticulum meshwork fibrils, vascular channels are much more abundant than usual, and vascular endothelium is hyperplastic.

Discussion

The ten lymph nodes included in this study were collected gradually over a period of 5 years. Seen individually and at extended intervals their variety was too great to impress upon the various observers any characteristic picture. When, however, the group was studied as a whole it became evident that there was sufficient underlying similarity to permit their ready distinction, not only from lesions observed in malignant lymphoma, but also from all but four of several hundred hyperplastic nodes which have passed through the laboratory in the same period of time. This characteristic picture was not produced by any single pathognomonic feature but by the general pattern of changes within the node.

Of prime importance in differentiation from malignant lymphoma was the maintenance, despite considerable distortion, of the nodal architecture. Both peripheral and radial sinuses could invariably be distinguished in at least portions of the node, although compression on the one hand and packing by proliferating lymphocytes and other mononuclear elements on the other frequently almost obscured them. Germinal centers were likewise generally identifiable but were variable in size and appearance. Lessened follicular prominence appeared to arise from a tendency, which became well marked in the nodes removed during later stages of the disease, for the borders of the greatly enlarged, hyperactive centers to become irregular and vaguely delimited. This was due partly to pseudopod-like projections of the center into surrounding pulp and partly to the encroachment upon the follicular borders of increasing numbers of active immature and atypical cells in the pulp itself.

In contrast to the pictures observed in the ordinary hyperplasias, three features were preeminent. First was the marked proliferative activity in the pulp which, as has been noted above, served to obscure the margins of the follicles (Fig. 7). The second was an extensive but distinctly focal proliferative activity of clasmatocytes, the cytoplasm of which became progressively more abundant and acidophilic until the appearance of "epithelioid cells" was simulated (Fig. 8). Although clustered to form small nodules they never showed a concentric tubercular arrangement and neither necrosis nor giant cells were ever noted.

The final and most nearly pathognomonic feature was the appearance throughout the pulp, on the edges of the germinal centers, and in the sinuses, of large numbers of the specific infectious mononucleosis (I.M.) cells (Figs. 2 and 5). It would be rash to claim that the appearance of a cell in fixed tissue sections was specific but with Zenker fixation and the phloxine-methylene blue stain these cells with their abundant, slightly foamy, cerulean blue cytoplasm are most conspicuous. Similar cells have not been observed in malignant lymphoma and but rarely in hyperplastic nodes. Impression and supravital preparations have shown them to be identical with the cells found in the circulating blood in this disease.

It seems worthy of repeated emphasis that this picture which was clearly evident in Zenker-fixed nodes stained with phloxine-methylene blue could be distinguished only with difficulty in material fixed in formalin or Bouin's fluid and stained with hematoxylin and eosin. Not only was it more difficult to recognize the persistent but distorted architecture in the latter instances, but the distinctive sky-blue color of the cytoplasm of the specific cells was not observed.

Once recognized, this lesion was so characteristic that independently several members of the laboratory staff recollected 3 similar cases and a 4th was revealed by a systematic search through the group of nodes catalogued under the heading of hyperplasia. In none of these 4 cases had infectious mononucleosis been diagnosed clinically, no blood smears were available for resurvey, and only 1 had a recorded differential count which was suggestive of the disease. In 2 cases 2 and 4 months after the time of the biopsies blood serum was obtained for heterophile

agglutination and both tests proved negative. Three individuals, one of whom had 43 per cent mononuclear cells in a blood smear, ran a benign clinical course, the lymph node swelling and symptoms disappearing spontaneously within a few weeks. The 4th patient, known to have had localized Hodgkin's sarcoma of the intestine which had been radically resected a year previously, was relieved of symptoms following X-ray therapy. A conclusive verdict ruling infectious mononucleosis either in or out is impossible on the basis of the available evidence. No unequivocal decision can be reached, therefore, regarding the specificity of the picture which has been described.

Summary and Conclusions

A survey of the literature reveals the fact that no consistent lesion has hitherto been described in the lymph nodes from patients with infectious mononucleosis. The authors have studied lymph nodes removed from ten such patients at various stages in the illness and have described a characteristic morphological pattern. This appears with such regularity in this disease and so rarely in other conditions that it is believed to have diagnostic importance.

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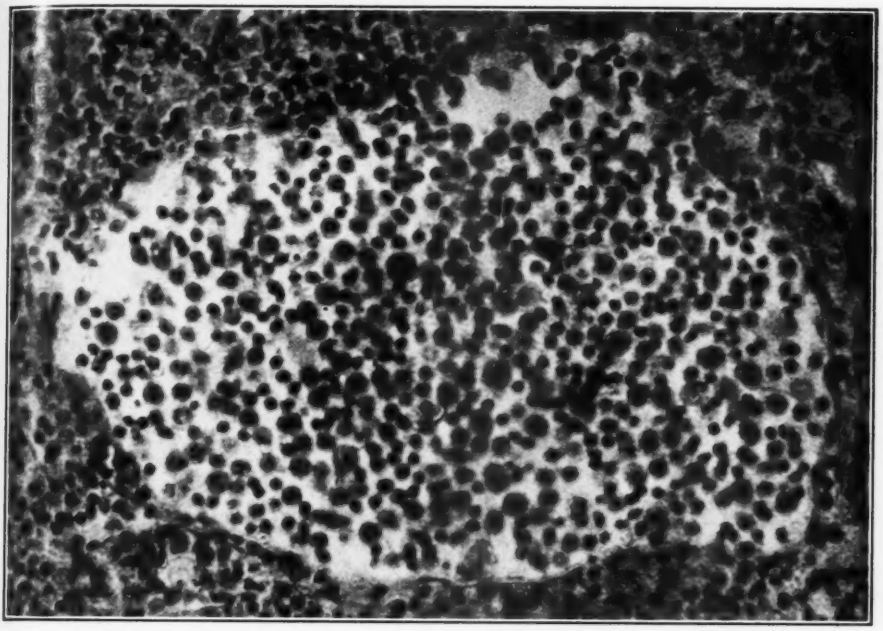
DESCRIPTION OF PLATES

PLATE 89

FIG. 1. A sinus distended by large numbers of lymphoid elements, a picture frequently seen in lymphatic leukemia. In infectious mononucleosis, however, there is a wide variety of cells evident (lymphocytes, clasmatocytes, I. M. cells, and so on). × 400.

FIG. 2. A higher power view of the cells shown filling the sinus in Figure 1. There is a wide morphological variation. Most of the large elements present are infectious mononucleosis cells, one of which may be seen in mitosis. × 1000.

FIG. 3. A lymphoid sinus containing large numbers of clasmatocytes evidently arising from sinus wall cells. Extension of these elements from the sinus into the pulp is also apparent. × 400.



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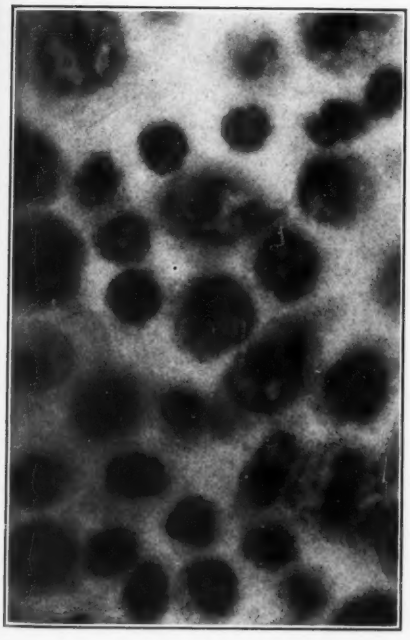

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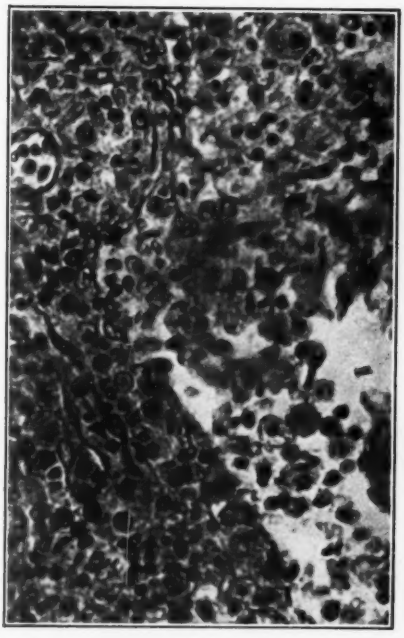
Gall and Stout



3

Lymph Nodes in Infectious Mononucleosis

PLATE 90

FIG. 4. A low power view of a lymph node exhibiting marked enlargement of follicles with prominent germinal centers. There is considerable variation in size and configuration. This phase is usually quite transient. × 50.

FIG. 5. A camera lucida drawing representing the four types of cells observed in the lymph nodes in infectious mononucleosis.

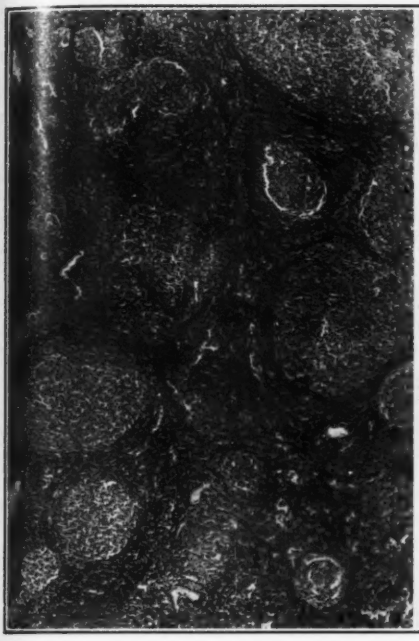
1 = A stem cell with abundant but vaguely outlined cytoplasm containing a large vesicular nucleus with prominent nucleoli but scanty reticular chromatin.

2 = Normal small lymphocytes.

3 = An infectious mononucleosis cell, the cytoplasm of which is coarsely granular in this instance. The nucleus is well chromatinized and eccentric in position. When stained with phloxine-methylene blue the cytoplasm has a mucin-like basophilic quality. × 1000.

4 = A clasmatocyte with abundant, finely granular cytoplasm and a reniform eccentric nucleus. In the stained preparation the cytoplasm is eosinophilic.

FIG. 6. An enormous germinal center is shown, composed mainly of infectious mononucleosis cells. There is marked thinning and irregularity of the lymphocytic collar as the result of encroachment by both germinal center and pulp elements. The border of the secondary nodule is now ill defined. × 400.



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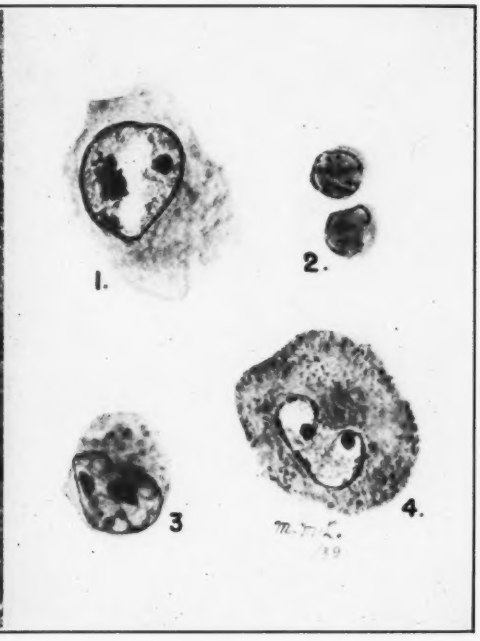

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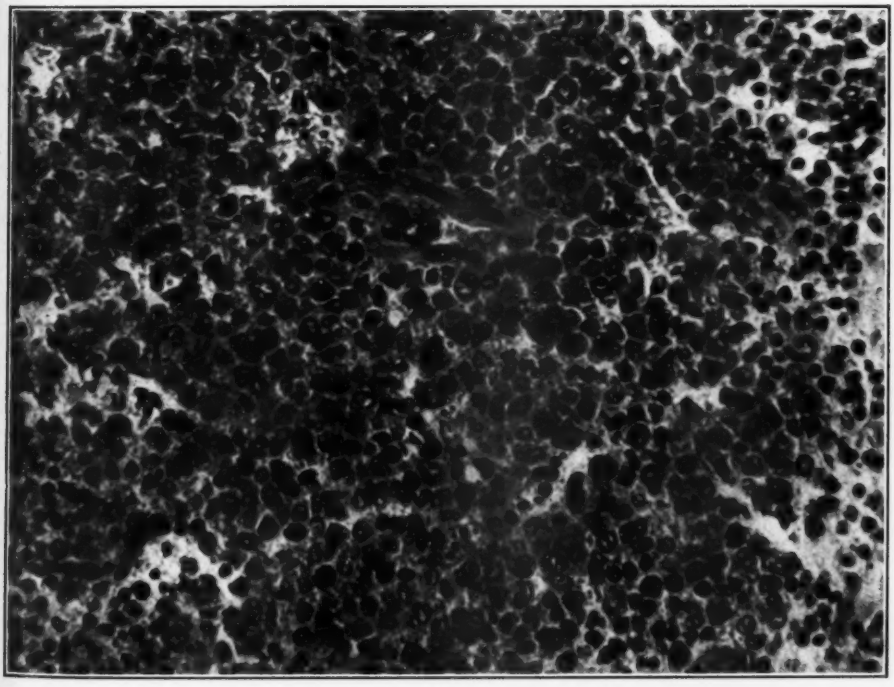

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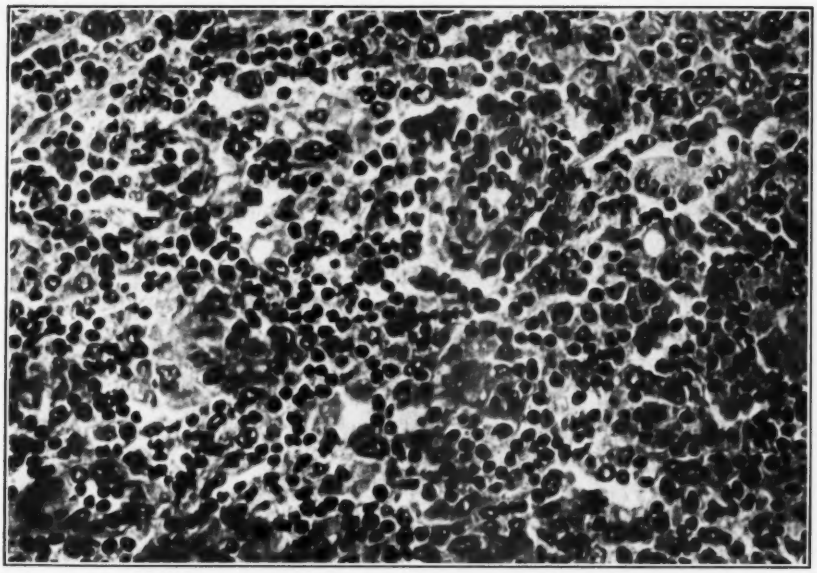
PLATE 91

FIG. 7. Lymph node pulp in the florid phase of the lesion demonstrating the marked interadmixture of lymphocytes, infectious mononucleosis cells, stem cells and clasmatocytes. Follicular arrangement can no longer be identified. × 400.

FIG. 8. An area in the vicinity of the periphery of a lymph node demonstrating marked infiltration of the pulp by clasmatocytes, many of which have assumed the appearance of "epithelioid cells." × 400.



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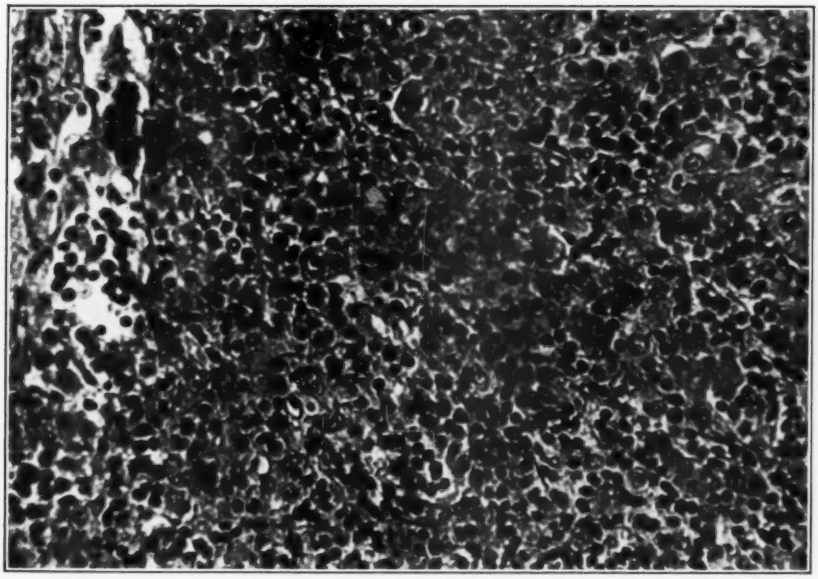

8

Gall and Stout Lymph Nodes in Infectious Mononucleosis

SPLENIC RETICULUM CELL TUMORS IN MICE *

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In a recent report [1] on the production of splenic tumors by the introduction of benzpyrene into the spleens of mice, attention was called to the rarity of splenic tumors in this species. Since that time 9 members of the inbred stock Ak, which has a high incidence of spontaneous leukemia, and 10 hybrids between mice of stock Ak and Rf, have died with splenic tumors. All of these tumors were benign, localized in the spleen, and appeared to be derived from modified fibroblasts which normally form the reticular framework of this organ. The tumor cells are obviously different from histiocytes (macrophages) which unfortunately are also often designated "reticulum" cells.

Literature on Splenic Tumors: Splenic tumors are rare both in man [2] and in animals.[3] The potentialities of the cells of the spleen are not well known and there is little knowledge concerning the spontaneous tumors arising in the fixed cells of this organ.

Friedreich [4] described the case of a man 56 years old with multiple tumors of the spleen and liver. These were composed of polygonal cells with spherical nuclei. The tumor cells in the spleen resembled liver cells of small and medium size so closely that Friedreich regarded them as derivatives of heterotopic liver tissue.

Weichselbaum [5] studied similar tumors but believed the neoplastic cells to be endothelial in origin.

The literature on splenic tumors of man has been reviewed by Smith and Rusk,[6] who collected 99 cases including 2 of their own. These were subdivided into 5 groups: (*a*) endothelial sarcoma, endothelioma and angiosarcoma (17 cases); (*b*) primary lymphosarcoma (21 cases); (*c*) primary sarcoma, round cell sarcoma and fibrosarcoma (29 cases); (*d*) carcinoma (19 cases); and (*e*) miscellaneous tumors (13 cases). In the latter group are included the case of Friedreich already mentioned and several

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different cases which the authors consider to be endotheliomas. The first group of splenic tumors is likewise not uniform. It includes benign tumors like those of Weichselbaum, as well as malignant forms of endothelioma with widespread metastases. The predominant cells of some of the latter resemble a transmissible neoplasm of mice to be described elsewhere.[7] Angiosarcoma is composed of well formed blood vessels containing erythrocytes and does not differ from similar tumors observed outside of the blood-forming organs.

In animals, primary splenic tumors are equally rare. Ball [8] described a growth in the spleen of a rabbit which he considered a benign endothelioma; the animal had similar lesions in the liver and lung. Guérin and Guérin [9] reported a malignant endothelioma of the spleen of a guinea pig with metastasis to the liver.

Data on Mice with Splenic Tumors: The splenic tumors here described were found among approximately 5000 mice of stock Ak and hybrids between mice of this stock and those of stock Rf. No tumors were observed in the pure Rf mice or in different stocks of which an approximately similar number was observed during this period.

Table I is a summary of salient data on the mice with splenic tumors. It shows that the splenic tumors observed were almost three times as common in male as in female mice. The Ak mice were from 6 to 14 months of age at the time of death, the hybrid mice from 9 to 17 months. One of the mice also had leukemia. In all but this mouse neoplastic changes were localized in the spleen and the uninvolved part of the spleen had the approximate dimensions of the spleens of normal mice of the same stock. All tumors were single, spherical and sharply demarcated (Fig. 1). They were pale red, slightly firmer than normal splenic tissue, and measured from 4 to 22 mm. in greatest diameter. Many of the tumors contained large areas of necrosis and small areas of hemorrhage.

Microscopic examination showed changes ranging from focal hyperplasia of reticular or endothelial stroma cells to unquestionable tumors. It is common to see in sections of lymph nodes and spleens of mice detached large mononuclear cells of the stroma as shown in Figure 10. The number of these cells is often greatly increased but they remain localized to the usual sites of reticular stroma cells and normal splenic tissue persists between them.

Such hyperplastic changes may be diffuse or focal. With increased proliferation of the reticular stroma cells the usual splenic elements between them disappear and the normal splenic tissue about them becomes compressed. The end stage of the process is a definite tumor, as illustrated in Figures 1 and 2. While the early

TABLE I

Data on Mice with Splenic Tumors

Number of mouse	Stock of origin	Sex	Age	Size of Spleen	Size of Tumor
			mos.	*mm.*	*mm.*
M 587	Ak	M	6	15 × 4	15
Aki 64	Ak	F	7	20 × 6	15
Akh 152	Ak	M	8	28 × 14	15
Akf 225	Ak	M	9	13 × 5	5
Akf 133	Ak	F	9½	12 × 4	10
Akh 217	Ak	M	10	22 × 16 × 14	22
Akg 80	Ak	F	12	22 × 6 × 3	20
Aki 192	Ak	M	13	12 × 4	7
Aki 152	Ak	F	13	12 × 4	6
Akf 90	Ak	F	14	15 × 5	4
Ha 821	Backcross to Ak	M	9	18 × 3	12
Ha 822	Backcross to Ak	M	11	12 × 5	20
Hf 509	Backcross to Ak	M	11	10 × 4	15
Ha 146	Backcross to Ak	M	13	24 × 8	20
Hf 968	Backcross to Ak	M	13	15 × 5	7
Ha 372	Backcross to Rf	M	9½	15 × 4	15
He 358	Backcross to Rf	M	12	7 × 5	8
Hb 753	Backcross to Rf	M	17	10 × 4	15
Hb 160	F_2	M	17	16 × 4	10

changes are those of hyperplasia, there is no sharp borderline between these hyperplastic and neoplastic alterations.

The microscopic appearance of the fully developed tumors is illustrated in Figure 2. The tumor cells are polygonal with finely granular, acidophilic cytoplasm and ill-defined cell borders. The nuclei are large, round or oval, with one, infrequently two, nucleoli. The cells occur in sheets and cords which are separated by endothelial-lined spaces containing blood. Large irregular areas

of hemorrhage and necrosis are conspicuous in most tumors. Masson's trichrome stain and Mallory's aniline blue stain for connective tissue show little stroma. Silver stains reveal scanty reticulum in the nodules, usually about the blood spaces, seldom between the tumor cells (Fig. 3). The microscopic appearance of another large tumor (Ha 822) is illustrated in Figures 4 and 5. Figure 4 shows the margin of the growth and compression of normal splenic tissue. Figure 5 is a higher magnification of the growth with tumor cells similar to those shown in Figure 2 but showing greater variation in size of nuclei than the cells in Figure 2. The cells have large nuclei with thick nuclear membranes, the cytoplasm is granular and acidophilic, and the cell borders are ill-defined.

There are foci of erythroblasts scattered throughout these benign splenic growths and, to a lesser extent, foci of myelocytes. Erythrogenic foci in a large tumor are illustrated in Figure 6. The tumor of the spleen measured 15 mm. in diameter, the remainder of the spleen 15 by 4 mm. Grossly the tumor resembled the splenic tumors already described. An attempt was made to transfer this growth (M 587) to normal mice but with no success.

Microscopic examination of the tumor (M 587) shows almost complete replacement of the splenic tissue by tumor. Normal splenic tissue is found only at the margin of the growth. A large part of the tumor is necrotic and hemorrhagic and the latter parts are separated from the viable tumor by a thick layer of young fibroblast-like cells. The bulk of the tumor is composed of polygonal cells which vary greatly in size and shape. Cords of epithelial-like cells are separated by endothelial-lined capillaries which are dilated in several areas. About the endothelial and epithelial-like cells are numerous polygonal cells that resemble Kupffer cells of the liver. Scattered throughout the tumor are numerous focal accumulations of erythroblasts and a few small groups of young myeloid cells. There are numerous detached tumor cells near a hematoma, some polygonal and epithelial-like, others elongated, and still others resembling young fibroblasts. There are transitional forms between fibroblast-like and epithelial-like tumor cells. The size of nuclei and nucleoli is variable in the epithelial-like cells, and many cells have very large nucleoli.

Mouse M 587 had been injected intravenously with a sus-

pension of neoplastic cells of the transmissible neoplasm Ha 202. Fragments of the splenic growth M 587 were injected into 10 mice of a stock susceptible to Strain Ha 202, but none had a tumor when killed 107 days later. Microscopic sections of the growth M 587 are indistinguishable from the benign splenic tumors described and the cells have no resemblance to those of the Strain Ha 202. The unsuccessful attempt at transmission and the microscopic appearance of the growth support the opinion that it was not produced by Strain Ha 202. This was the only benign splenic tumor found among approximately 140 mice of stock Ak and Ak hybrids that had been injected with cells of Strain Ha 202.

The early changes in the development of splenic tumors consisting of focal hyperplasia with formation of a nodule measuring 4 mm. in diameter are illustrated in Figure 7.

Approximately 75 per cent of the mice of pure stock Ak die of spontaneous leukemia and the disease is common though less frequent among the hybrids of this stock. Yet only 1 of the 20 mice (Akg 80) here described with spontaneous benign splenic tumors had leukemia also.

Mouse Akg 80, Lymphoid Leukemia with Early Splenic Tumor: A large part of the tumor is necrotic and the necrotic tissue is invaded by normal fibroblasts. The well preserved part of the tumor is composed of anastomosing young spindle shaped or polygonal cells (Figs. 8 and 9) which in several fields resemble closely the normal reticular stroma cells (Figs. 10 and 11) of lymph nodes. Groups of lymphocytes of medium size are seen scattered through the growth. The nodule also contains occasional small foci of erythroblasts. The structure of a lymph node of this mouse examined is obliterated by an overgrowth of lymphocytes of medium size. Similar cells are present in large numbers in the portal areas and scattered diffusely throughout the liver. The large blood vessels show a slightly increased number of lymphocytes. The pancreas is adherent to the spleen and the adhesions are infiltrated by masses of lymphocytes.

Discussion

Histological Characteristics of Splenic Tumors in Mice: The framework of the normal splenic pulp is composed of fibroblast-like and endothelial cells supported by delicate reticulum.

Scattered throughout the pulp are numerous histiocytes (macrophages). In tissue cultures there is a conspicuous difference between histiocytes on the one hand, and endothelial and fibroblast-like cells on the other; in fixed preparations, forms that appear transitional are numerous and it is commonly assumed that the fibroblast-like or endothelial cells are precursors of histiocytes.

The morphological characteristics of the neoplastic cells of the splenic tumors here described are like those of hypertrophic reticular stroma cells and have no resemblance to histiocytes. The nuclei are large and vesicular, with one or several nucleoli, and never lobed or indented. The cells are polygonal or slightly elongated, resembling small liver cells, but similar cells are common in normal lymph nodes (Figs. 10 and 11). The tumor cells form cords separated by sinusoids lined by normal endothelial cells. Neoplastic cells do not line the capillaries of the growth. Endothelioma produced in chickens by virus occasionally assumes cuboidal forms,[10, 11] but these cells are in continuity with normal endothelium lining the sinusoids.

It is unlikely that the tumor cells originate in heterotopic liver cells as suggested by Friedreich. Figure 10 shows that detached reticular cells of normal lymph node may be polygonal and assume a superficial resemblance to liver cells. It is unlikely that the tumor cells are mesothelial because the microscopic appearance of the early tumors indicates that the neoplastic cells originate in the stroma.

The origin of tumors in focal hyperplasia is a question of fundamental importance. Many of the changes observed are definitely those of focal or diffuse hyperplasia of reticular stroma cells, while others are those of frank tumors. Intermediate forms are so numerous as to warrant the assumption that these benign neoplasms represent the end stage of a hyperplastic process and that they do not originate from misplaced epithelial cells.

Extramedullary Myelo- and Erythropoiesis: Extramedullary myelo- and erythrogenesis is not uncommon in older mice but it is relatively infrequent in Stock Ak and, if present, is inconspicuous. The normal part of the spleen of mice bearing these tumors contained almost no foci of myelopoiesis, whereas the early tumors were thickly spotted with them. The microscopic appearance of the tumors composed of epithelial-like neoplastic cells with scat-

tered blood-forming foci resembles that of human embryonal liver. It is possible that these neoplasms are favorable sites for blood formation or that the changes leading to the formation of these tumors stimulate myelogenesis. The latter opinion is supported by the observation that the early tumors contain many blood-forming foci, the largest almost none.

Summary

Nineteen cases of benign splenic tumors have been observed in a stock of mice (Ak) that has a high incidence of spontaneous leukemia, and in its hybrids.

The neoplastic cells are probably derived from reticular stroma cells of the spleen. The changes observed in different mice range from focal hyperplasia to definite tumors.

The small tumors contain scattered foci of myelo- and erythrogenesis which are absent or less conspicuous in the normal part of the same spleen.

Note: Miss Jean Brundage rendered valuable technical assistance during the course of this study.

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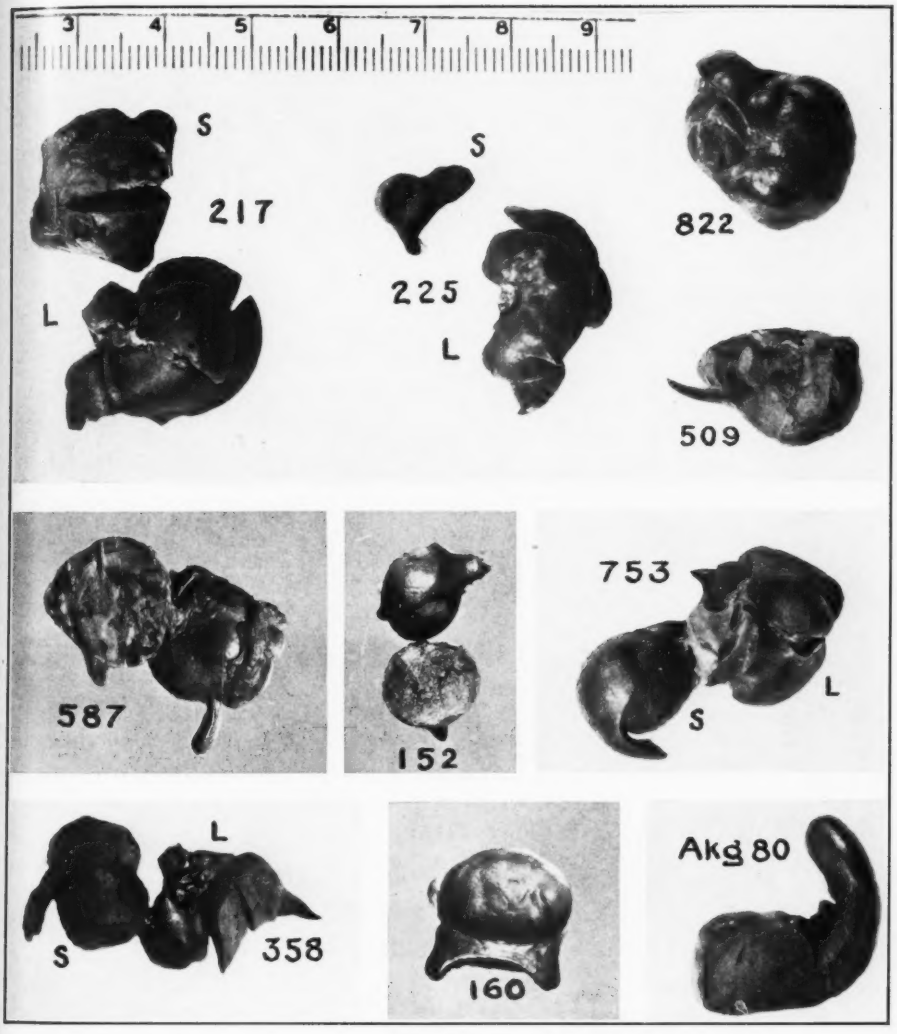
DESCRIPTION OF PLATES

The sections were stained with hematoxylin and eosin, unless otherwise stated. The magnifications are approximate.

PLATE 92

FIG. 1. Gross photographs of 9 of the splenic tumors described. The livers of several mice are shown for comparison to illustrate the relative size of the tumors. S = spleen. L = liver. The numbers refer to the number of the tumor-bearing mouse. The spleen of a leukemic mouse (Akg 80) with a lymphoid tumor is shown for comparison.

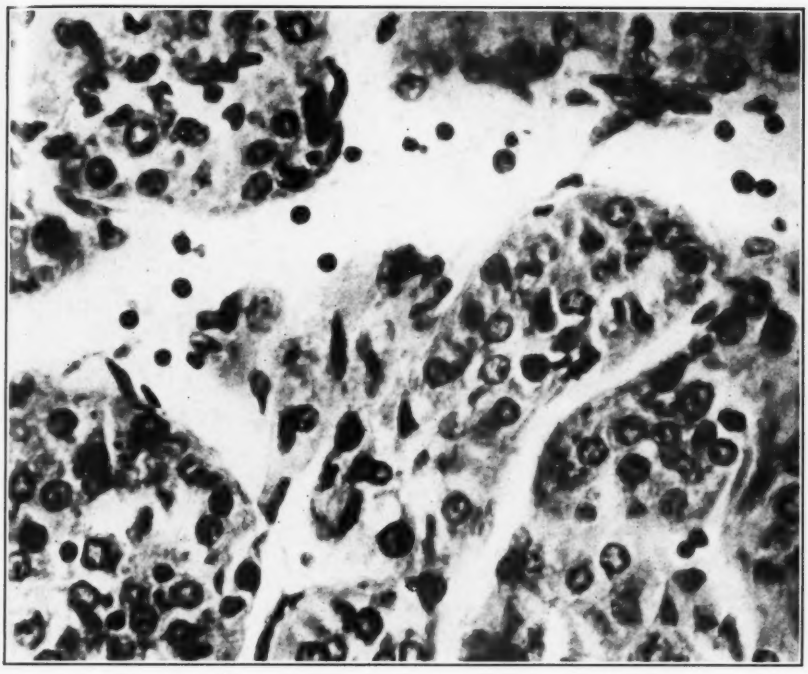


1

PLATE 93

FIG. 2. Splenic tumor Akh 217. There are columns of cuboidal tumor cells between endothelial-lined sinusoidal capillaries. × 500.

FIG. 3. The same tumor stained with silver for reticulum according to the technic of Foot. × 250.



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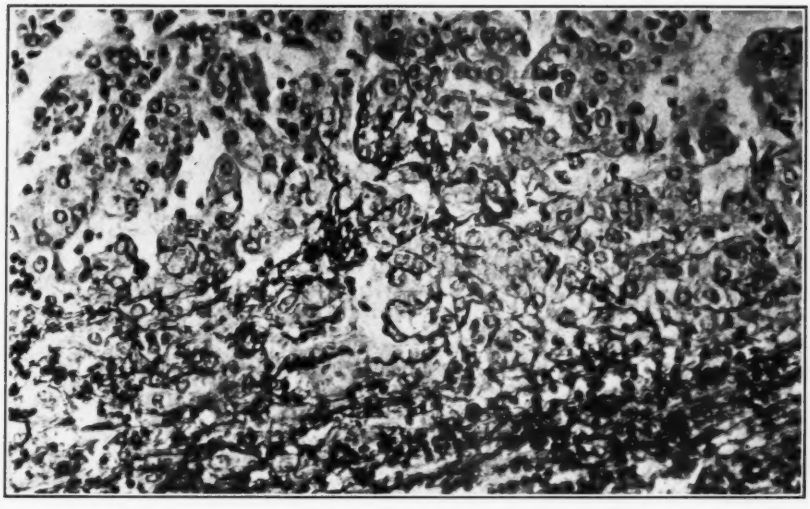


3

Furth and Krumdieck Splenic Reticulum Cell Tumors in Mice

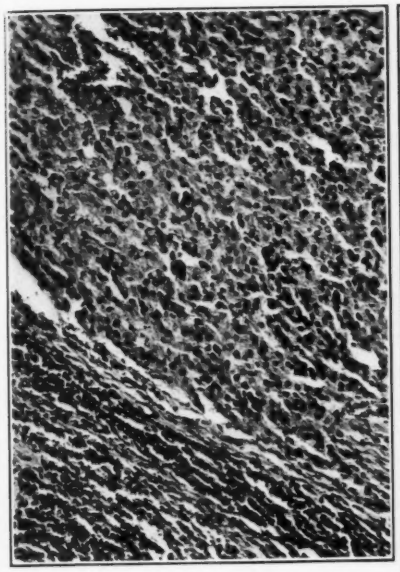
PLATE 94

FIG. 4. Splenic tumor Ha 822. Margin of the growth with compressed normal splenic tissue. × 150.

FIG. 5. Higher magnification of tumor cells shown in Figure 4. × 300.

FIG. 6. Splenic tumor M 587. Erythrogenic foci are present among the tumor cells. × 300.

FIG. 7. Splenic nodule in mouse Akf 90 showing focal hyperplasia of large mononuclear stroma cells.



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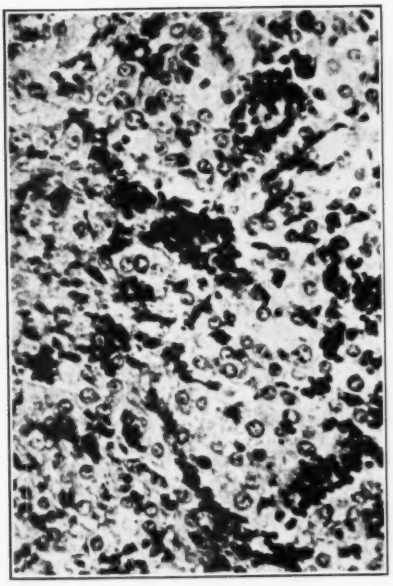

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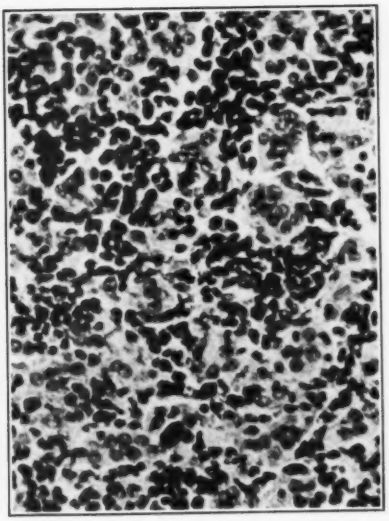


7

Furth and Krumdieck

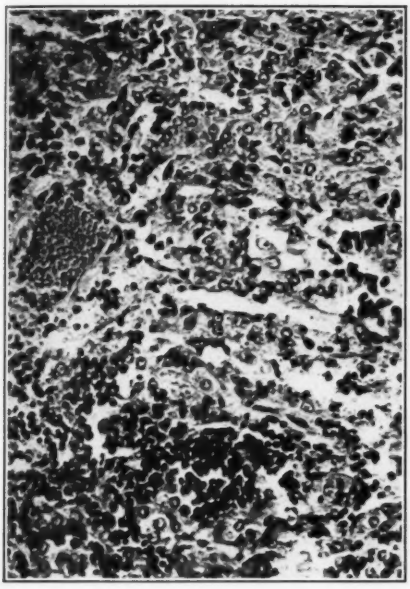
Splenic Reticulum Cell Tumors in Mice

PLATE 95

FIGS. 8 and 9. Splenic tumor Akg 80. There are foci of lymphoid infiltrations. Figure 8 × 150, Figure 9 × 250.

FIG. 10. Large detached mononuclear cells in the medulla of a lymph node of a normal mouse. × 600.

FIG. 11. Lymph node of a normal cat showing, in reticular arrangement, mononuclear cells similar to those illustrated in Figure 10 and to those which form the tumors (Figs. 2–9). × 600. (This section was kindly loaned to us by Dr. J. Nonidez for the demonstration of normal reticular stroma cells in the lymph node.)



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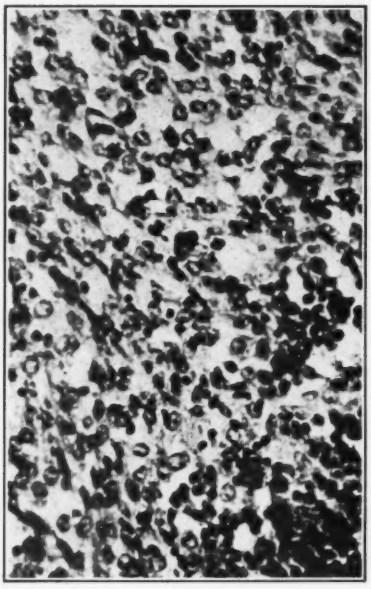

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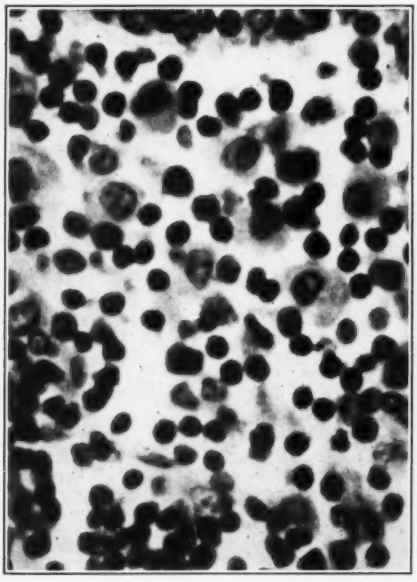

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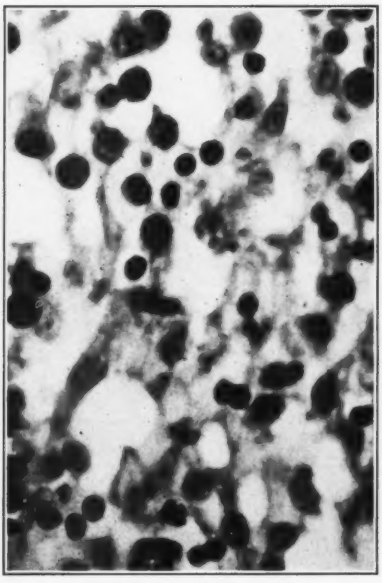

11

Furth and Krumdieck Splenic Reticulum Cell Tumors in Mice

A TRANSMISSIBLE MALIGNANT NEOPLASM OF MICE ORIGINATING IN RETICULAR OR ENDOTHELIAL CELLS *

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The benign neoplasms of the spleen described in a preceding communication [1] originated in either specialized endothelial or reticular stroma cells of that organ. All of these tumors occurred in mice of stock Ak or in their hybrids. In 1 hybrid female mouse (No. Ha 202) whose son had a benign splenic tumor, a malignant neoplasm was observed which appears to have originated in the same cell type. This neoplasm has been transmitted in serial passages, and presents several unusual features as follows:

(*a*) The malignant cells are slightly phagocytic like those of the endothelial cells of the spleen. (*b*) *In vitro* they resemble large epithelioid forms of macrophages (histiocytes). (*c*) Although the mouse with the spontaneous disease had advanced infiltrations in the liver, spleen and bone marrow, after subcutaneous inoculation the neoplastic cells seldom infiltrate these organs and after intravenous injection usually remain localized in the lung. (*d*) The subcutaneous tumor produced by these cells is characterized by extensive hemorrhage and necrosis, and is associated with profound anemia and advanced erythropoiesis in the spleen. (*e*) Like the leukemias arising in corresponding hybrid mice, this neoplasm is readily transplantable to mice of the ancestral leukemic stock and first generation hybrids, but it does not grow at all in the ancestral non-leukemic stock. (*f*) The cells readily produce large tumors on the chorioallantoic membrane of the chick. These unusual characteristics induced us to describe this neoplasm.

Mouse Ha 202 was 16 months old when its abdomen became distended and both liver and spleen were readily palpable. The

* These investigations have been supported by grants from the Lady Tata Memorial Trust, the International Cancer Research Foundation, and the Jane Coffin Childs Memorial Fund.

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lymph nodes were not felt. The number of leukocytes appeared normal and the differential count was: polymorphonuclears 60 per cent, metamyelocytes 15 per cent, lymphocytes 22 per cent, and monocytes 3 per cent. The presence of numerous basophilic erythrocytes in blood smears indicated profound anemia; the erythrocytes were of approximately normal size and shape and there were no nucleated erythrocytes.

The mouse was killed and was found to be pregnant. The spleen was firm and grayish red, and measured 4.5 by 1.5 by 1 cm. Subcutaneous, mediastinal and para-aortic lymph nodes were not enlarged. The liver was greenish gray and slightly enlarged. On its surface were numerous yellowish gray areas about 1 mm. in diameter. The region of the mesenteric lymph node was occupied by a gray mass measuring approximately 2.5 by 1.5 by 1 cm. (Fig. 1).

Microscopic examination showed that the spleen was almost completely replaced by malignant cells and occasional small foci of lymphocytes remained. There were a few areas infiltrated by myeloid cells with metamyelocytes predominating. Scattered small foci of erythrogenic cells and a few megakaryocytes were present. The neoplastic cells were closely packed and cell boundaries were not evident. The cytoplasm of the malignant cells was acidophilic and slightly granular. Most of the nuclei were oval but they varied in shape from almost spherical to elongated forms simulating the letter J. There were from one to four large nucleoli and small areas of chromatin condensation. Mitotic figures were present in moderate numbers. In less crowded areas the cells varied greatly in size and shape, and cells with more than one nucleus were present in small numbers (Fig. 4).

In the liver were numerous foci of cells similar to those described in the spleen. Many Kupffer cells were laden with golden yellow pigment. A few small groups of myeloid cells, mostly metamyelocytes, were scattered throughout the organ. The tumor in the mesentery was composed of the malignant cells described above. No evidence of lymphoid tissue remained although the mass probably represented growth in a mesenteric lymph node. The bone marrow showed conspicuous myeloid hyperplasia with metamyelocytes predominating. There was moderate erythrogenesis. In several areas there were small groups of cells similar to those

described in the spleen, and about them erythrogenic cells were numerous.

Transmission Experiments

Fragments of the spleen of mouse Ha 202 produced tumors in 5 of 9 related mice that were injected subcutaneously. Four of 8 mice in the 2nd passage similarly injected, and 7 of 8 in the 3rd, developed subcutaneous tumors. Salient data on the results of transmission experiments are given in Table I.

Table I

Transmission Experiments

Stock of mice	Number of experiments	Number of mice	
		Injected	+
Ak	5	29	24 (83%)
Rf	3	30	0 (0%)
F_1 (Ak × Rf)	3	23	17 (74%)
Backcross (F_1 or F_2 × Ak)	17	82	73 (89%)
Backcross (F_1 or F_2 × Rf)	2	16	2 (13%)
Sl	3	20	0 (0%)
F_1 (Ak × H)	5	30	30 (100%)

Ak = leukemic stock; Rf = non-leukemic stock; Sl = inbred stock of mice not related to stocks Ak and Rf; and H = stock C_3H of C. C. Little.

The table shows that the tumor is readily transmissible to the mice of its ancestral leukemic stock (Ak) but not to mice derived from its non-leukemic ancestor (Rf). The percentage of successful inoculations of this neoplasm in hybrid mice derived from these two stocks is similar to that of leukemias arising in corresponding hybrids.[2] Almost every member of the F_1 generation is susceptible; hence, susceptibility is dependent on dominant factors though the number of factors involved is uncertain.

Anatomical Characteristics

The subcutaneous tumors produced were red, soft, and resembled splenic pulp. They were spotted with numerous areas of necrosis (Fig. 5). They often penetrated by continuity the wall of the chest and abdomen (Fig. 7) and formed masses in the peritoneal cavity which were slightly firm and usually attached to the omentum but rarely invaded the spleen and liver. In the pleural

cavity they produced a spongy growth and in both cavities there was a hemorrhagic exudate. In a few instances there were metastases in the regional lymph nodes (Fig. 9). Microscopic examination of the tumor showed scanty development of blood vessels and few fibroblasts. A silver stain for reticulum showed a few delicate reticulum fibers among the tumor cells. In sections stained with aniline blue few collagenous fibers were seen. The malignant cells resembled those in the mouse with the spontaneous disease. In many cells the nucleus was eccentric and there was a light staining zone in the central part of the cell (Fig. 3).

After intravenous injection, red spongy tumors occupied the lung (Fig. 2), the alveoli were filled with blood, and there was hemorrhagic effusion in the pleural cavities, but the spleen, liver and lymph nodes remained free from metastases. In 1 mouse injected intravenously malignant cells invaded the bone marrow and erythrogenic cells were conspicuous about the tumor cells.

The mice became pale soon after the development of the subcutaneous growth and the anemia appeared to be far more intense than one would expect from hemorrhage alone. Chart 1 indicates values for red blood cells in mice with subcutaneous tumors of this strain.

The chart shows that the development of anemia was concurrent with the growth of the tumor. Microscopic examination of the spleens of mice with subcutaneous tumors showed advanced erythrogenesis (Fig. 6) and slight myelogenesis. There were a few erythrogenic foci in the liver and the bone marrow showed advanced erythrogenic and myelogenic hyperplasia.

Observation on the Malignant Cells Grown In Vitro

Margaret R. Lewis and George O. Gye have been successful in growing these cells *in vitro* on coverslips and in roller tubes fastened to the glass by means of a trace of chicken plasma and bathed in a mixture of Tyrode's solution, human blood serum and beef extract. We are indebted to them for most of the following data.

In vitro the rate of reproduction of the cells is unusually high. The malignant cells resemble epithelioid forms of monocytes. Their diameter as measured in fixed and stained preparations varies from 7 to 41 μ but most cells are very large, comparable to

the large epithelioid forms of monocytes (Figs. 12 and 13). These malignant cells are sluggish and barely move. They have an undulating membrane similar to that of monocytes (Fig. 13). Most cells have a single spherical nucleus with multiple nucleoli.

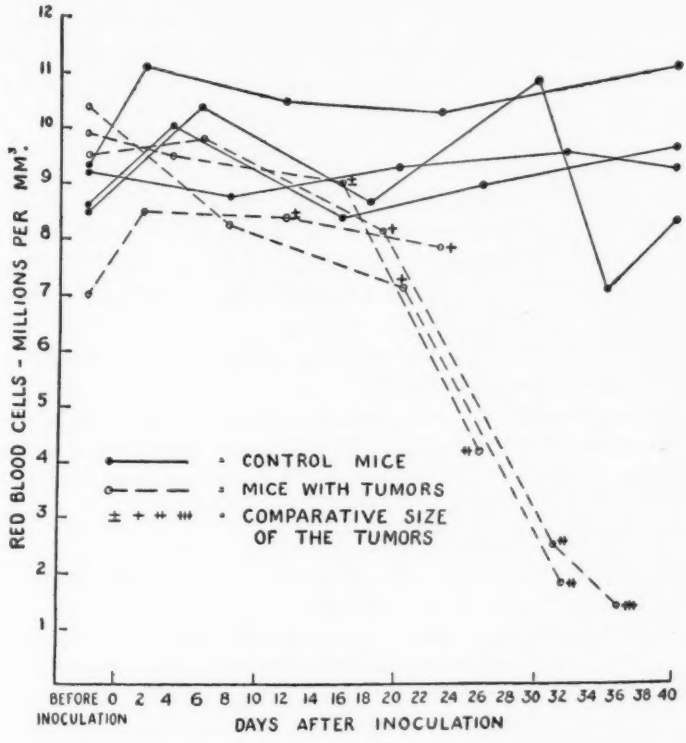

CHART 1

The nucleus is very large, the nuclear membrane sharp. The nucleoli are sharply defined and very large. Margaret R. Lewis * has observed in cultures of this growth the formation of giant cells by division of nuclei and failure of cytoplasm to divide (Fig. 13). She has also noted chromosomal disturbances similar to those described by W. H. Lewis as characteristic for malignant cells.

In cultures the nuclei are surrounded by an unusually large

* Personal communication.

number of mitochondria, more so than normal blood cells, but these structures fail to take up Janus green. The cells exhibit the phenomenon of pinocytosis (ingestion of fluid), a phenomenon described by W. H. Lewis.

The cells do not ingest particles of India ink injected intravenously, but *in vitro*, on direct contact, a few carbon particles appear in the cytoplasm while mononuclear phagocytes in the same cultures contain many ingested particles.

Growth of the Malignant Cells on the Chorioallantoic Membrane

As early as 1911 Rous and Murphy [3] reported that tumors can be grown in developing mammalian embryos, although with difficulty. A transmissible avian sarcoma was grafted on developing hen's eggs with greater ease. The scarcity of reports concerning the growth of mammalian tumors on the chorioallantoic membrane of the chick may indicate that attempts of many investigators have been unsuccessful.

Of five attempts to grow different human neoplasms and eight attempts to grow different neoplasms of mice on the chorioallantoic membrane, only two of the latter were successful. One of these was the malignant tumor here described which grows luxuriantly on this membrane.

In the first experiment the malignant cells of strain Ha 202 were grown in 15 successive passages.

Small fragments of tumor tissue measuring 2 to 3 mm. in diameter were implanted on the chorioallantoic membrane of chicken eggs following the technic described by Burnet.[4] Subpassages were made usually at weekly intervals. During a period of from 7 to 9 days the minute implanted fragment of tumor tissue reached a size of from 6 to 12 mm. in diameter. Of the 6 mice that were injected with tumor fragments from the 3rd passage, 4 developed progressively growing tumors. In a second experiment 4 and in a third 6 successive passages were made on the chorioallantoic membrane. In the latter experiment all of 4 mice injected with tumor particles from the membrane of the 3rd passage and 1 of 4 mice injected with growth of the 4th passage on the membrane developed tumors.

Figure 10 shows tumors collected from the chorioallantoic

membrane 7 days after implantation. Approximately 50 per cent of the eggs yielded tumors of similar size; in the remaining 50 per cent the membranes either showed no growth or, in a few instances, the growth stopped with or without death of the embryo. Microscopic examination showed the remarkable picture of capillaries distended with fowl erythrocytes among the tumor cells which are indistinguishable from those grown in mice (Fig. 11) and show numerous mitotic figures.

Discussion

Character of the Neoplastic Cell: The interrelationship of various forms of monocytes and their relation to endothelial cells and fibroblast-like reticular cells of the blood-forming organs is obscure. The characteristics of neoplasms arising in these cells are likewise not sufficiently known to differentiate these cells from each other and from related cells, *e.g.*, mesothelial cells. Whereas in the earlier literature the term endothelioma was used to include most ill-defined neoplasms that resembled some forms of endothelial cells, there is now a tendency to group under the name of monocytoma tumors composed of large mononuclear cells of unknown character.

The similarity of the mononuclear neoplastic cells of strain Ha 202 to those of epithelioid forms of monocytes leads one to consider that the two are related. The following characteristics make this possibility unlikely: The nuclei of these malignant cells are very large, and have a sharp nuclear membrane and large conspicuous nucleoli. The nuclei are approximately spherical and unlike those of monocytes show no tendency to become indented or lobed. With supravital stains "rosettes" characteristic of monocytes are not evident and the cells are not actively phagocytic. The opinion that the malignant cells of strain Ha 202 are related to reticular or endothelial cells of the spleen or lymph nodes is supported by the following observations: (*a*) The occurrence of the spontaneous neoplasm Ha 202 in a stock of mice in which benign reticular tumors of the spleen occur.[1] (*b*) The son of mouse Ha 202 had such a tumor. (*c*) The main sites of localization of the spontaneous disease were spleen, liver and mesenteric lymph node. (*d*) The neoplastic cells are similar to the detached large mononuclear cells in sinuses of blood-forming organs.

Genetic Constitution of the Malignant Cells: The neoplasm originated in a mouse that was a hybrid between a highly leukemic stock (Ak) and a non-leukemic stock (Rf). Previous studies [2] have shown that the spontaneous leukemias arising in similar hybrid combinations behave in transmission experiments like those arising in the pure leukemic stock. They can be transmitted to most members of the pure leukemic stock (Ak) but to none of the non-leukemic stock (Rf). The transmissible neoplasm here described is in this respect indistinguishable from leukemias occurring in this hybrid combination. The non-malignant splenic tumors described in a previous communication [1] were found only in Ak mice and their hybrids and not in Rf mice.

Anemia Associated with this Neoplasm: Tumors of this strain measuring 1 cm. or more in diameter were associated with a profound anemia and advanced erythrogenesis in the spleen. Since this growth produces no distant hemorrhages, and internal metastases but rarely, the hemorrhagic character of the growth itself does not seem sufficient to account for the anemia. Neither is it likely that the anemia is secondary to the extensive necrosis occurring in the tumor because necrosis of even greater degree observed in different spontaneous and transmitted tumors is not associated with so profound an anemia. The occurrence of extramedullary myelopoiesis in association with transmissible tumors has been described by Jaffé,[5] M. R. Lewis,[6, 7] and others, but we are not familiar with any report on erythrogenesis in association with transmissible tumors.

Summary

A transmissible neoplasm in mice is described and evidence is presented that the neoplastic cells are related to reticular or endothelial stroma cells of the spleen and lymph nodes.

The cells grow well in the subcutaneous tissue and invade adjacent tissue by continuity. After intravenous introduction they produce large tumors in the lung and occasional small infiltrations in the bone marrow. The tumor growth is accompanied by profound anemia and extreme erythropoiesis in the spleen. The cells are not actively phagocytic but on contact with India ink *in vitro* a few carbon particles appear in the cytoplasm.

In transmission experiments the malignant cells of this neo-

plasm behave like those of the leukemias arising in hybrid mice; both can be transplanted to almost every mouse of the ancestral leukemic stock but to no mouse of the ancestral non-leukemic stock.

The malignant cells grow readily on the chorioallantoic membrane of chicks. On microscopic examination these neoplastic cells are indistinguishable from those grown in mice and the capillaries among them are filled with chicken erythrocytes.

NOTE: Miss Mary Boon rendered valuable technical assistance during the course of this study.

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DESCRIPTION OF PLATES

The tissues illustrated were fixed in Zenker-formalin or in Bouin's solution. The sections were stained with hematoxylin and eosin. The magnifications are approximate.

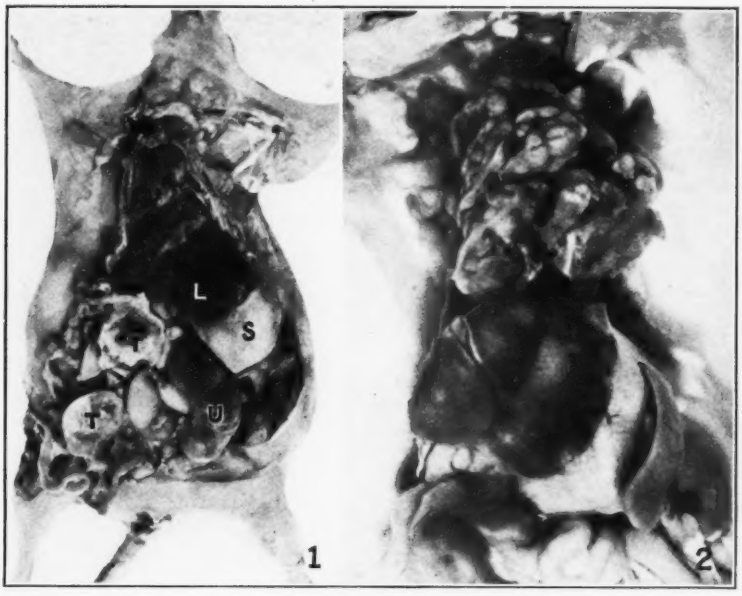
PLATE 96

FIG. 1. Mouse Ha 202, showing part of the enlarged spleen (S), mesenteric tumor (T), tumor nodules in the liver (L), and uterine horn (U) containing fetuses.

FIG. 2. Tumors in lungs following intravenous inoculation of malignant cells. The liver, spleen and lymph node are free from grossly evident infiltration.

FIG. 3. Tumor in the lung. The malignant cells vary in size and many contain a central area of lightly staining cytoplasm. × 270.

FIG. 4. A multinucleated tumor giant cell. × 675.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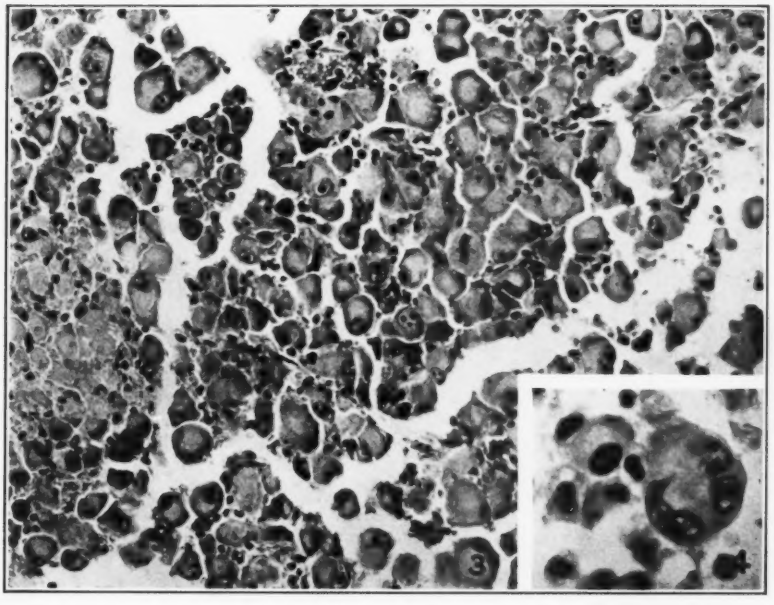
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PLATE 97

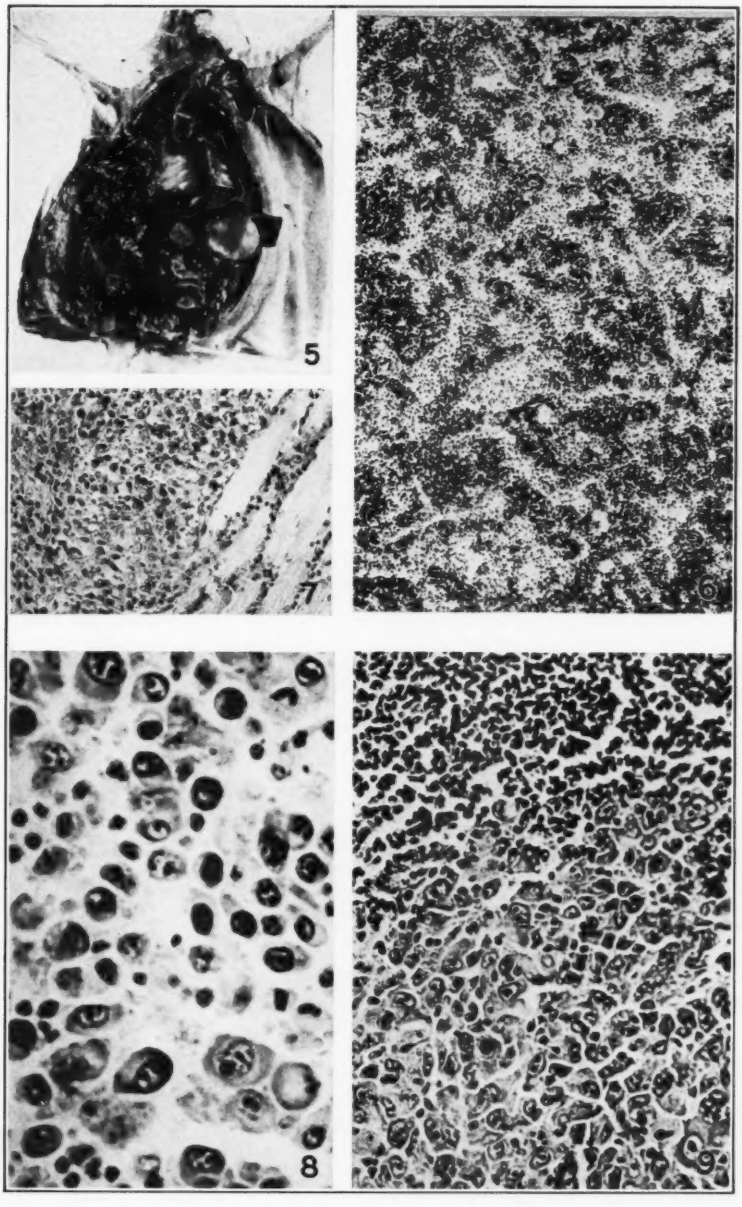
FIG. 5. Tumor in the thoracic and abdominal walls following subcutaneous inoculation of malignant cells. The lungs, liver and spleen contain no metastases.

FIG. 6. Spleen of mouse bearing subcutaneous tumor, showing advanced erythrogenesis. There are no malignant cells. × 100.

FIG. 7. Malignant cells of the subcutaneous tumor invading muscle of the abdominal wall. × 150.

FIG. 8. Malignant cells of subcutaneous tumor. × 675.

FIG. 9. Lymph node, draining the region of a subcutaneous tumor, infiltrated by malignant cells. × 270.



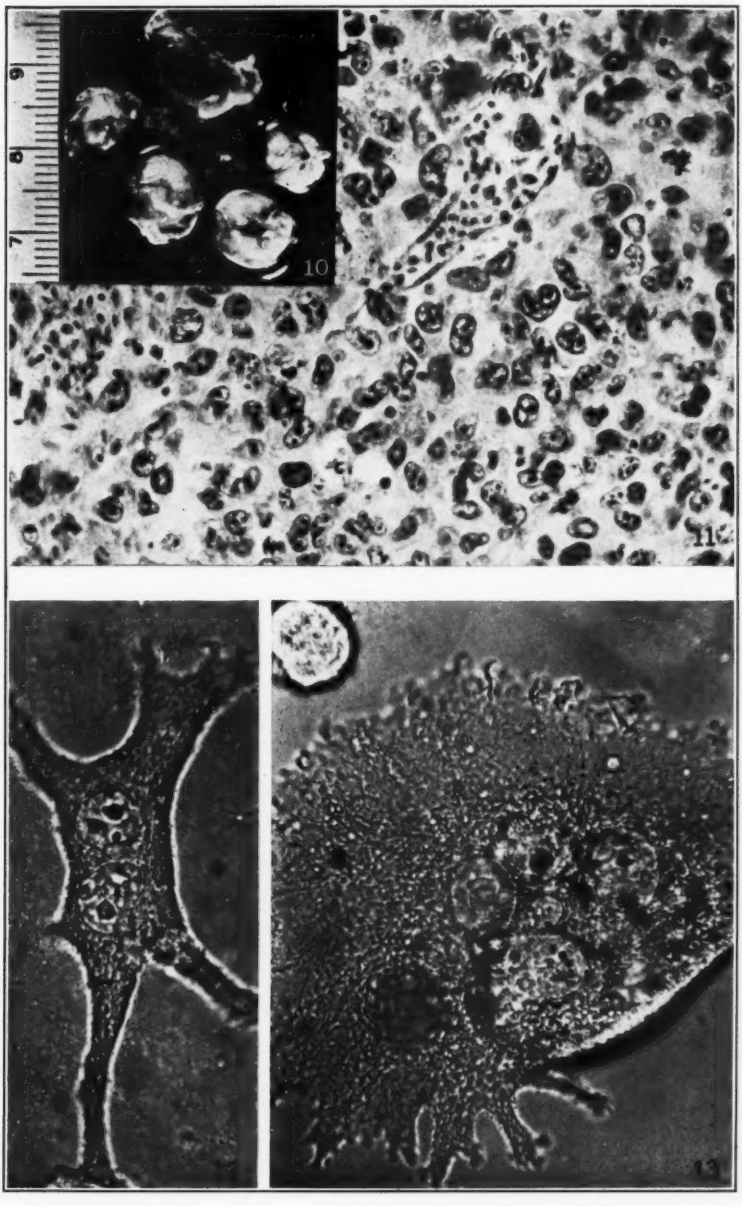
Barnes and Furth Transmissible Malignant Neoplasm of Mice

PLATE 98

FIG. 10. Tumors grown on the chorioallantoic membrane of the chick.

FIG. 11. Microscopic section of tumor grown on the chorioallantoic membrane. Two sinusoids are filled with avian erythrocytes and are surrounded by malignant mouse cells with several mitoses. × 450.

FIGS. 12 and 13. Malignant cells in the living state. Multiple nuclei and nucleoli, undulating membrane and mitochondria are illustrated. × 1000. We are indebted to Dr. Margaret R. Lewis for these figures.



Barnes and Furth Transmissible Malignant Neoplasm of Mice

PRIMARY ENCEPHALOMYELITIS IN GOATS ASSOCIATED WITH LISTERELLA INFECTION *

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Bacterial infections of the nervous system generally take the form of a meningo-encephalitis or of focal metastatic encephalitis clearly secondary to infection elsewhere in the body. Primary encephalitis, from which a specific bacterium may almost constantly be recovered, raises new problems in neuropathology and warrants detailed consideration.

The bacterium in question, belonging to the genus *Listerella*, has a wide host range. It was first isolated in 1926 by Murray, Webb and Swann [1] from rabbits which were sick with mononucleosis. The name *B. monocytogenes* was applied. Independently Pirie [2, 3] in South Africa recovered a similar organism from a native rodent, the gerbille. Because the lesions were predominantly in the liver, he designated the bacterium as *Listerella hepatolytica*. The generic name *Listerella* has been finally adopted for this class of organisms.

That this new genus was of importance in human pathology soon became apparent. Burn,[4, 5, 6] and Schultz and his coworkers [7, 8] independently recovered unusual bacteria from human cases of meningitis. At first the identification was difficult, but finally conclusive evidence was brought forward to classify them with the *Listerella* group. Other papers [9, 10, 11] have since appeared in the literature reporting new cases of meningitis in which the same organism was found. It is probable that the cases reported by Atkinson [12] in 1917 were of the same type.

In addition, this bacterium has been recovered from human cases of infectious mononucleosis.[13, 14] Schmidt and Nyfeldt [15] have reported a series of 5 cases in which infectious mononucleosis and meningitis have coexisted. In 4, spinal fluid cultures revealed *Listerella*. In 1, there was also a positive blood culture.

As yet, in human nervous system involvement, only meningitis

* Received for publication January 7, 1940.

has been attributed to the *Listerella*. In lower animals, however, many cases of encephalitis have been observed from which the organism was recoverable in pure culture. Jones and Little [16] reported sporadic encephalitis in cows. A comparable disease in sheep has been studied by Gill,[17, 18] by Jungherr,[19] and by Biester and Schwarte.[20] TenBroeck (unpublished) observed similar cases in sheep and goats. In addition, TenBroeck (also unpublished) recovered *Listerella* from chickens which showed no nervous system involvement, but instead myocardial necrosis.

Biological and immunological properties of *Listerella* have been studied by Seastone,[21] and by Webb and Barber,[22] and Barber,[23] as well as by other authors to whom reference has been made. These aspects of the problem will not be considered at the present time.

Material

The purpose of the present communication is to describe in detail the pathological findings in a series of 9 goats which were first studied by TenBroeck and Seastone in 1936. Although their observations have not been published, the pathological material has been placed at my disposal, as well as the clinical and bacteriological reports. The following data are drawn from their unpublished records.

These 9 animals all came from a single farm in New Jersey. They were taken ill during January, February and March, 1936, and brought to this laboratory for examination. During the same period several other goats of the same flock died with comparable symptoms, but were not autopsied or studied from the bacteriological standpoint.

The clinical course of the disease, lasting 2 or 3 days, showed nothing distinctive. The animals were obviously sick, with moderate fever, and reacted as in any severe infection. They would be down, unable to rise, but specific obvious neurological signs were rare. Specific motor weakness or spasticity was noted only infrequently.

Of the 9 animals studied, pure cultures of a Gram-positive organism were obtained from the brains of 6. In 1 other, the blood gave a positive culture although the brain did not. In 2 animals all cultures were negative. The organisms recovered were identified as belonging to the genus *Listerella*. Since the ani-

mals all came from a single flock and since the clinical course and the pathology were the same in every instance, there can be no reasonable doubt that the animals with negative cultures suffered from the same disease.

Paraffin embedded blocks of various portions of the nervous system, as well as of other organs, were obtained. For study hematoxylin-eosin and toluidine blue stains were made, and appropriate methods for demonstration of myelin, axis cylinders, reticulum and bacteria were employed. Because of the absence of unembedded tissue, fat stains could not be done.

Pathology

In the central neuraxis the lesions are restricted almost exclusively to the brain stem, especially the medulla and spinal cord. The disease process is much milder in the midbrain, while in the thalamus merely a cuffed blood vessel may occasionally be found. The basal olfactory regions may rarely show slight involvement. The neocortex and cerebellum are not affected.

The typical lesion consists of a small, compact, focal collection of cells which may be situated in white or gray matter. Examples in the spinal cord and medulla are seen under low power in Figures 1 and 2. These foci vary considerably in size. In serial sections cut at 15 μ, single collections of cells may vary from 0.075 mm. to over 1 mm. in greatest dimension. Although the smaller foci are approximately spherical, those that extend over many sections are asymmetrical, the greatest dimension or length (usually running rostrocaudally) ranging up to ten times the cross section.

When examined under higher power the composition of these foci can be studied. Usually, though not always, there are a few polymorphonuclear leukocytes scattered sparsely among the other elements, but the majority of cells are always mononuclear. In favorable situations several different cell types may be clearly recognized. Numerous microglia may be identified by the abundant chromatin and irregular shape of their nuclei as well as by their typical branching cytoplasm, excellently brought out by toluidine blue. Even more numerous are the microglia-like cells, probably derived from the vascular adventitia. Typical oligodendroglia, with round compact nuclei having dense conspicuous

chromatin and with scanty cytoplasm, are also clearly recognizable. Very prominent are larger, pale, oval or kidney shaped nuclei with scanty chromatin content. Such cells are provided with a moderate amount of compact cytoplasm, frequently round or oval, sometimes slightly angular, or with small, blunt, tuberous processes. These may be called epithelioid cells, for they are comparable to similar cells observed in well recognized granulomatous lesions of the brain. Astrocytes, easily recognizable in routinely stained sections of the nervous system, are not a component of these lesions.

In Figures 5 and 6 are seen examples of such circumscribed nodules under moderate enlargement. The different types of nuclei are apparent although the cytoplasmic details are not very clear in the reproduction. Also, in Figure 5 may be seen the syncytium formed by the cells and characteristic of many of these nodules. This syncytium is apparently similar to what Lillie [24] has described in the brain in Rocky Mountain spotted fever cases. These small foci or granulomas are usually in close association with blood vessels although serial sections are necessary to establish the relationship. With such series it can be observed that the vessels may pass either directly through the mass of cells, or are in contact with it on one side. In the larger foci followed serially, more than one blood vessel can be seen in close apposition, and frequently the cell mass is larger in proximity to the vessels, smaller in the intervening regions. Sometimes, however, in spite of serial sections, a vascular relationship cannot be determined for every focus.

In many instances, especially where the cellular foci are less compact, a streaming of cells from the adventitia of blood vessels can be seen. Such streaming is also observable in relation to pial surfaces of the nervous system. The cells involved are mononuclear and show a considerable range of morphology. Some are similar to the so-called "active" and "transitional" microglia. Others have large vesicular nuclei and resemble the epithelioid cells.

The blood vessels show considerable alteration. Pronounced perivascular cuffing is readily observable in the low power illustrations, and may even occur in portions of the brain stem where focal nodules are absent. The coats of the blood vessels are pro-

foundly disturbed. The endothelium may be swollen and proliferated. At other times it is degenerated and hyalinized. Small thrombi are not infrequent. The media may show marked disorganization and infiltration, with practically no structure remaining except infiltrating cells in an irregular hyaline matrix. Polymorphonuclear as well as mononuclear cells occur in the infiltrations. Figure 7 illustrates the disorganization of structure and infiltration of one vessel. It is noteworthy that perivascular hemorrhages have not been observed.

Where the disease process is advanced the parenchyma shows not only vascular and focal pathology, but also more diffuse and confluent changes. Rarely there may be small abscess formation, where dense masses of polymorphonuclear leukocytes, mingled with a few mononuclear cells, replace tissue that is practically destroyed. More typically, however, the separate foci become quite intense and the intervening tissue is more lightly infiltrated. Figure 3 shows under low power a Nissl stain of the medulla of 1 animal. Separate foci are large and there is moderately dense tissue infiltration that is diffuse. Such infiltration, however, is predominantly mononuclear and composed of the same types of cell that are found in the nodules. Figure 11 shows such a diffuse tissue infiltration under higher power. In such instances the parenchyma is not too severely damaged, incomparably less so than in the small abscesses. Figure 4 is a myelin stain of the section adjacent to that shown in Figure 3, and taken at the same magnification. There are several, small focal areas of myelin destruction corresponding to the intense focal collections of cells. But the diffuse tissue infiltration (Fig. 11) is accompanied only by a mild lightening of the myelin, observable chiefly on the upper part of the figure.

Foci of demyelination show interesting features when examined under higher power. Figure 9 is taken from a myelin stained cross section of the spinal cord and shows a portion of the lateral column. In the center of the figure the clear space corresponds to a focal collection of cells such as is shown in Figure 5. Remnants of myelinated fibers persist, with here and there large ballooned sheaths. These ballooned fibers correspond in Figure 5 to the round spaces surrounded by cytoplasmic rims. The axis cylinders are present in these demyelinated areas to a slightly greater extent

than the persisting myelin sheaths. In general the impression obtains that the fibers are pushed aside and choked out by the compact mass of cells, rather than undergoing any acute active or primary destruction.

The nerve cells in affected areas may or may not show changes. The striking feature is the paucity of morphological alterations that are visible in spite of the close proximity of large focal infiltration. But at times the nerve cells may be severely damaged or necrotic. In Figure 11, in the upper right corner, is one such injured neuron, with severe central loss of Nissl granules, but without swelling. Other cells, not figured here, may show more typical severe cell disease of Nissl. Increase of satellite glia cells is frequent, and numerous examples have been observed of necrotic nerve cells being actively phagocytosed by polymorphonuclear leukocytes. Pictures such as Figures 3 and 4 from a paper by Hurst,[25] taken from a case of virus encephalitis, could be accurately reproduced from the present goat material. Nuclear inclusions have not been observed.

In the majority of cases bacteria may be demonstrated somewhere in the diseased tissues, although by no means in every separate lesion. The morphological character of the bacteria as they appear in fixed tissue is adequately dealt with by previous workers and need not be repeated here. Figure 8 illustrates a marked degree of bacterial invasion. This particular animal was killed by chloroform and autopsied immediately, so that there was no opportunity for postmortem growth. In other lesions and in other animals fewer bacteria would be demonstrable in a field of comparable size. In a single oil immersion field of any given inflammatory or granulomatous lesion, bacteria, if present, would usually range up to a dozen in number, sometimes free in the tissue, usually phagocytosed by mononuclear cells. Bacteria were never demonstrable in the endothelium or walls of the blood vessels.

Meningeal involvement by the inflammatory process is common, but is generally slight in extent and secondary to parenchymal damage. Much less common are areas of meningitis where the underlying parenchyma is essentially free of lesions. Such an instance is seen in the lower part of Figure 10.

The disease process is not limited to the central nervous system

or the leptomeninges. In one instance (Fig. 10) a well marked nodule was present in the dura over the spinal cord. Unfortunately, most of the sections do not include the pachymeninx, so that the frequency of this locus cannot be fairly estimated. That it may occur, however, is noteworthy.

In the peripheral nervous system lesions are seen occasionally in the cranial ganglia or nerves. Figure 12 illustrates a portion of the gasserian ganglion and associated fifth nerve fibers in one instance. Several other similar examples are available, although such peripheral location is by no means constant. In the illustration there is an intense infiltration of the body of the ganglion, chiefly by mononuclear cells. The infiltration is largely sharply focal and nodular, although the process is diffuse in some areas. The ganglion cells themselves show surprisingly little evidence of damage. Most noteworthy, however, are the focal nodules in the nerve bundles. Under higher power these cell collections are indistinguishable from similar nodules in the cord and medulla, already described above.

Apart from the nervous system there is no constant pathology, although abnormalities are found. Nematodes in the lung are probably not related to the disease picture. Of greater relevance is the frequent involvement of the liver, either in the form of fatty infiltration or, less frequently, scattered focal necrosis. In 3 instances the kidneys show a definite glomerulitis. Cardiac abscesses were not noted.

Discussion

The distribution of lesions is essentially restricted to the brain stem, especially the medulla and the spinal cord. The neocortex is entirely spared. Not only is there no injury to the parenchyma, but even the overlying meninges are not affected. So far as can be determined the cerebellum too is free of lesions, although blocks of this organ were not available in every instance. There are no data bearing on the cause of this predilection for the brain stem.

The primary lesion in the parenchyma is a circumscribed focal collection of mononuclear cells (with or without an admixture of a few neutrophilic leukocytes) in close relation to a blood vessel. The vessels show marked degenerative change, coupled with in-

filtration of the walls and adventitia. Diffuse cellular infiltration of nerve parenchyma and the occasional formation of small abscesses are secondary as well as late phenomena.

The small granuloma-like lesions and the blood vessel changes suggest a similarity to the lesions seen in the brain in typhus,[26, 27] Rocky Mountain spotted fever,[24, 28, 29] and toxoplasmosis.[30, 31] This bacterial encephalitis is, however, much more fulminating and is accompanied by vastly more perivascular infiltration. But the focal granulomatous lesions are more similar to Rickettsial encephalitides than to virus infections of the nervous system.

Organisms of the genus *Listerella* have a very wide host range in nature and produce a variety of unrelated diseases. Thus, necrosis of liver and spleen, cardiac abscesses, meningitis, encephalitis and mononucleosis occur in different hosts, while the causative organism in each case is very closely related. Experimentally all these separate conditions can be reproduced, with the exception of encephalitis.

In reference to encephalitis, the causal rôle of the organisms is still somewhat obscure. Although the specific bacterium may be recovered from the brain in pure culture, Koch's postulates cannot be fulfilled, since the disease is not accurately reproducible by inoculation. In the cases reported here, TenBroeck (unpublished) injected pure cultures of *Listerella* intracerebrally into sheep and goats. The inoculated animals died, but the disease process was a meningitis and meningo-encephalitis, not a true encephalitis as has been described above.

In the literature similar difficulties are reported. Gill,[18] who isolated bacteria from the brains of sheep, on re-inoculation intracerebrally into normal sheep could produce only meningo-encephalitis. Inoculation into sheep by intracarotid injection or intranasal instillation similarly produced meningitis. Whatever brain involvement was present was not comparable to the naturally occurring disease, although suggestive evidence was obtained after intracarotid injection.

Jungherr,[19] attempting the experimental induction of the natural disease in sheep, was also unsuccessful. In mice, in a few instances, after nasal instillation of the bacteria, he was able to find typical nodules in the medulla, but he concludes that the pathogenesis of

the natural disease is not understood, although *Listerella* must play an important rôle as an etiological agent. On the other hand, Biester and Schwarte [20] believe that they reproduced the natural disease by inoculation, but they present no evidence on the subject.

Burn,[5] with organisms isolated from human cases of meningitis, produced purulent meningitis in rabbits after intravenous inoculation. Of 4 monkeys injected intravenously, 2 showed meningitis alone, a 3rd animal exhibited marked infiltration of the parenchyma of the cortex with polymorphonuclear leukocytes, while the 4th animal was not affected. This single instance of parenchymal involvement in a monkey is most unusual, for it resembles neither the human disease nor the encephalitis in lower animals.

Factors of host peculiarity are excellently brought out by Burn. Intravenous inoculation of *Listerella* into rabbits results in meningitis. Similar inoculation into guinea pigs causes myocardial abscesses without affection of the meninges. But intraperitoneal injection into guinea pigs results in meningitis without myocardial abscesses.

Gibson [9] inoculated rabbits and guinea pigs with *Listerella* isolated from human meningitis but was not able to reproduce either encephalitis or meningitis. Similarly Seastone,[21] working with organisms recovered from chickens, could not induce primary meningitis or encephalitis in rabbits or guinea pigs by intravenous injections. However, after profound systemic infection the nervous system showed a slight reaction.

It is clear from the literature that experimental meningitis can be produced quite regularly by subdural injections of *Listerella*, as well as less constantly by other routes of inoculation. Encephalitis, however, has not been satisfactorily demonstrated by experimental methods in spite of occasional suggestive instances. Obviously there is some additional factor in the naturally occurring cases of encephalitis which eludes experimental analysis. The suggestion has been made that in addition to the bacteria a virus is involved in the pathogenesis, so that the natural encephalitis is a mixed infection. However, attempts to demonstrate a separate virus have not been successful. The problem for the present remains unsolved. In the natural disease, in goats as well as in sheep and cows, the invasion of the brain parenchyma by bacteria ap-

pears to be a primary process, independent of any discoverable focus of infection elsewhere in the body.

Summary

In a series of 9 goats spontaneous encephalitis associated with *Listerella* infection was observed.

Pathologically the lesions were essentially restricted to the brain stem, especially the medulla and spinal cord. Peripheral nerves and ganglia were inconstantly affected. There was an associated meningitis.

The primary parenchymal lesion is a circumscribed focal collection of mononuclear cells (with or without an admixture of a few neutrophilic leukocytes) in close relation to a blood vessel. The infection is very fulminating. Diffuse tissue infiltration may supervene, sometimes with the formation of small abscesses, but these are secondary and late phenomena. The blood vessels show marked degenerative changes, coupled with cellular infiltration of the walls and adventitia. Bacteria can frequently be demonstrated histologically in the parenchymal lesions. Nerve cells may be destroyed, but there is relatively little tissue necrosis.

Data on experimental transmission and pathogenesis are briefly discussed. The similarity to Rickettsial encephalitides and cerebral toxoplasmosis is pointed out.

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DESCRIPTION OF PLATES

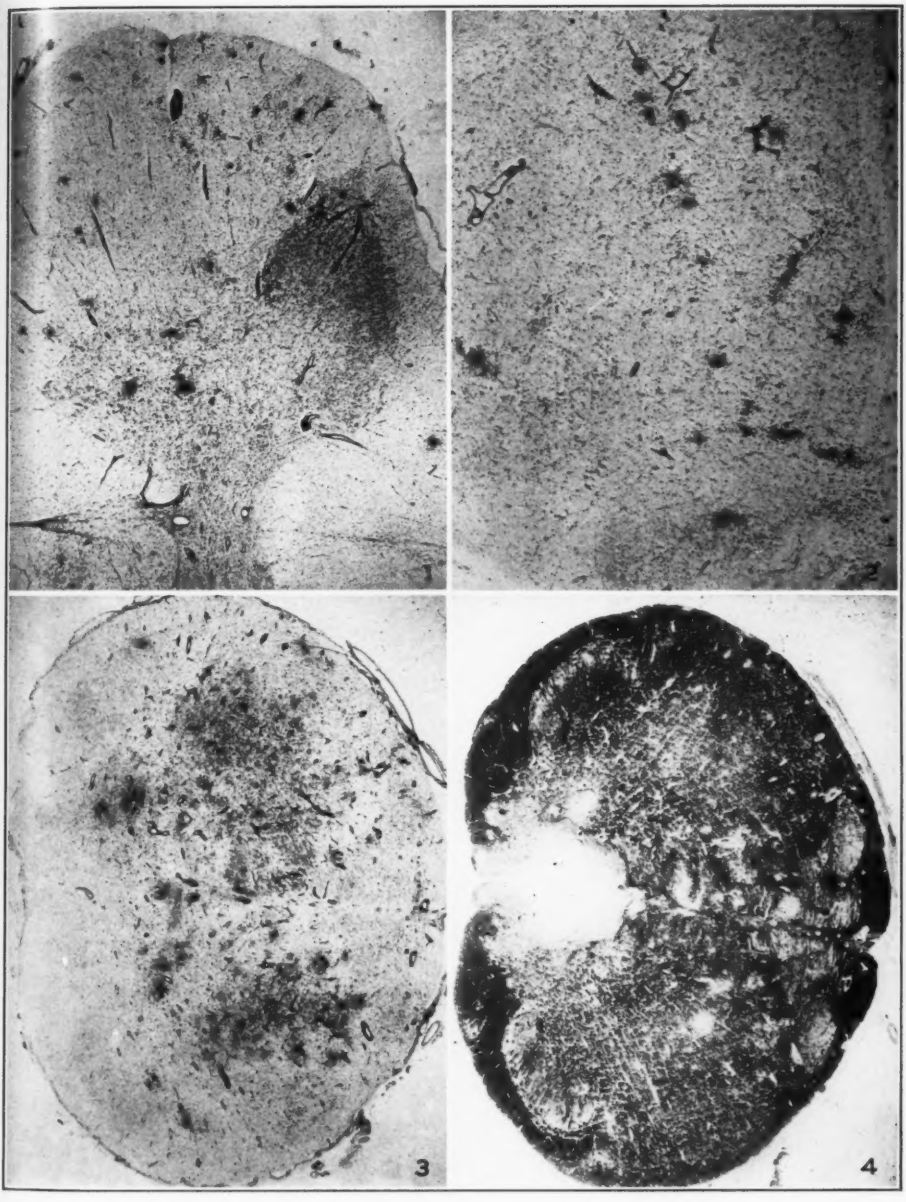
PLATE 99

FIG. 1. Spinal cord in the early stage of the disease process with numerous focal collections of cells in both white and gray matter. The blood vessels are quite heavily infiltrated. Toluidine blue stain. × 17.5.

FIG. 2. Medulla oblongata in the early stage of the disease process, with discrete inflammatory nodules, many of which are in obvious relation to blood vessels. Toluidine blue stain. × 32.

FIG. 3. Cross section of the medulla in the late stage of the disease. The focal cell collections are intense and large, and there is considerable diffuse parenchymal infiltration. The blood vessels are very heavily cuffed. There are a few necrotic areas. Meningitis is insignificant. Toluidine blue stain. × 8.

FIG. 4. Section adjacent to that in Figure 3, but stained for myelin. The heavily cuffed blood vessels and the focal areas of necrosis stand out as clear spaces. The severe diffuse infiltration of tissue seen in Figure 3 is reflected only by a mild lightening in the myelin stain. × 8.



King Encephalomyelitis in Goats

PLATE 100

FIG. 5. A focal collection of cells seen under higher power. There are a few polymorphonuclear leukocytes, but the majority are mononuclear, some glial, some derived from the blood stream or the vascular adventitia. A cytoplasmic syncytium can be seen. The large round spaces with cytoplasmic rims correspond to ballooned myelin sheaths. Toluidine blue stain. × 455.

FIG. 6. Another focal nodule, showing nuclei of epithelioid cells in addition to other elements. Toluidine blue stain. × 574.

FIG. 7. A blood vessel showing severe disorganization, necrosis and hyalinization, and marked infiltration with mononuclear and polymorphonuclear cells. Hematoxylin-eosin stain. × 550.

FIG. 8. Focus of Gram-positive bacteria in the parenchyma of the medulla. Gram-Weigert stain. × 1157.

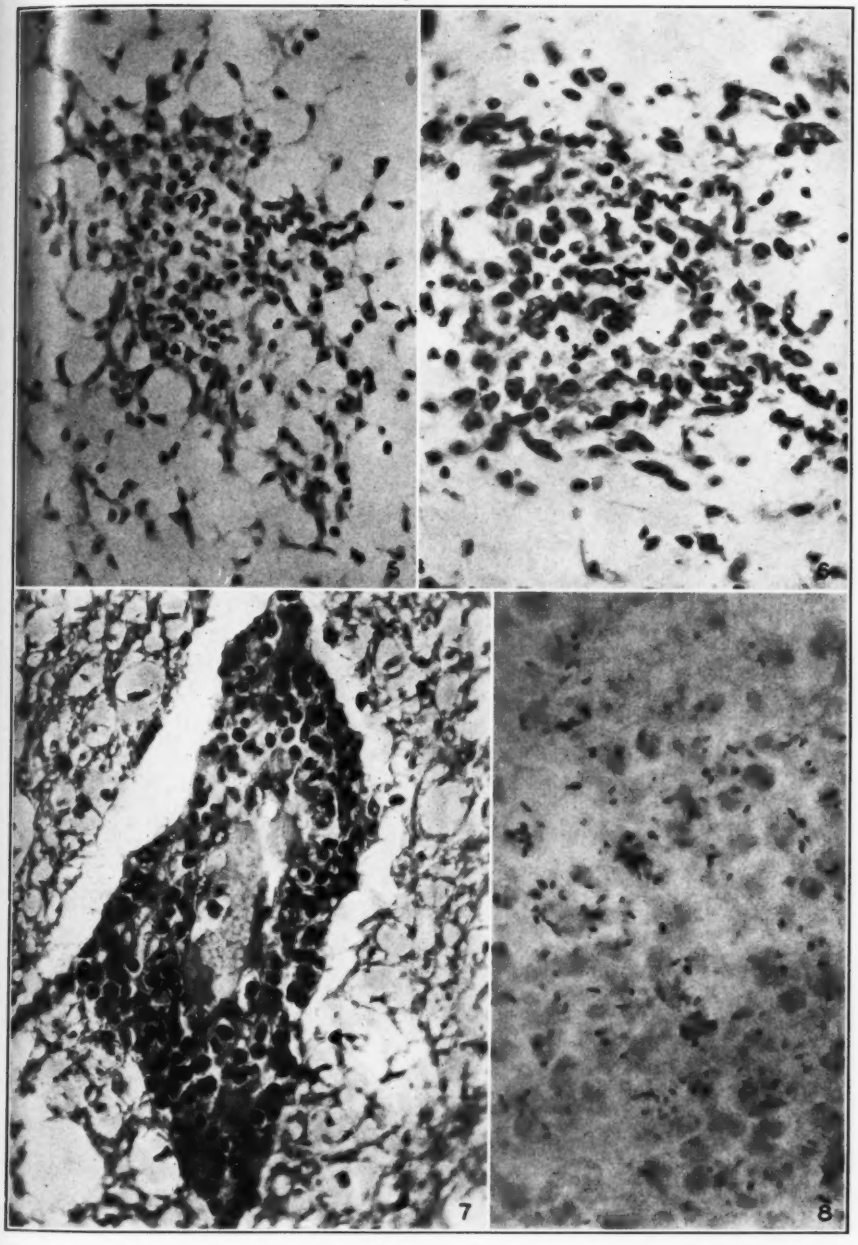

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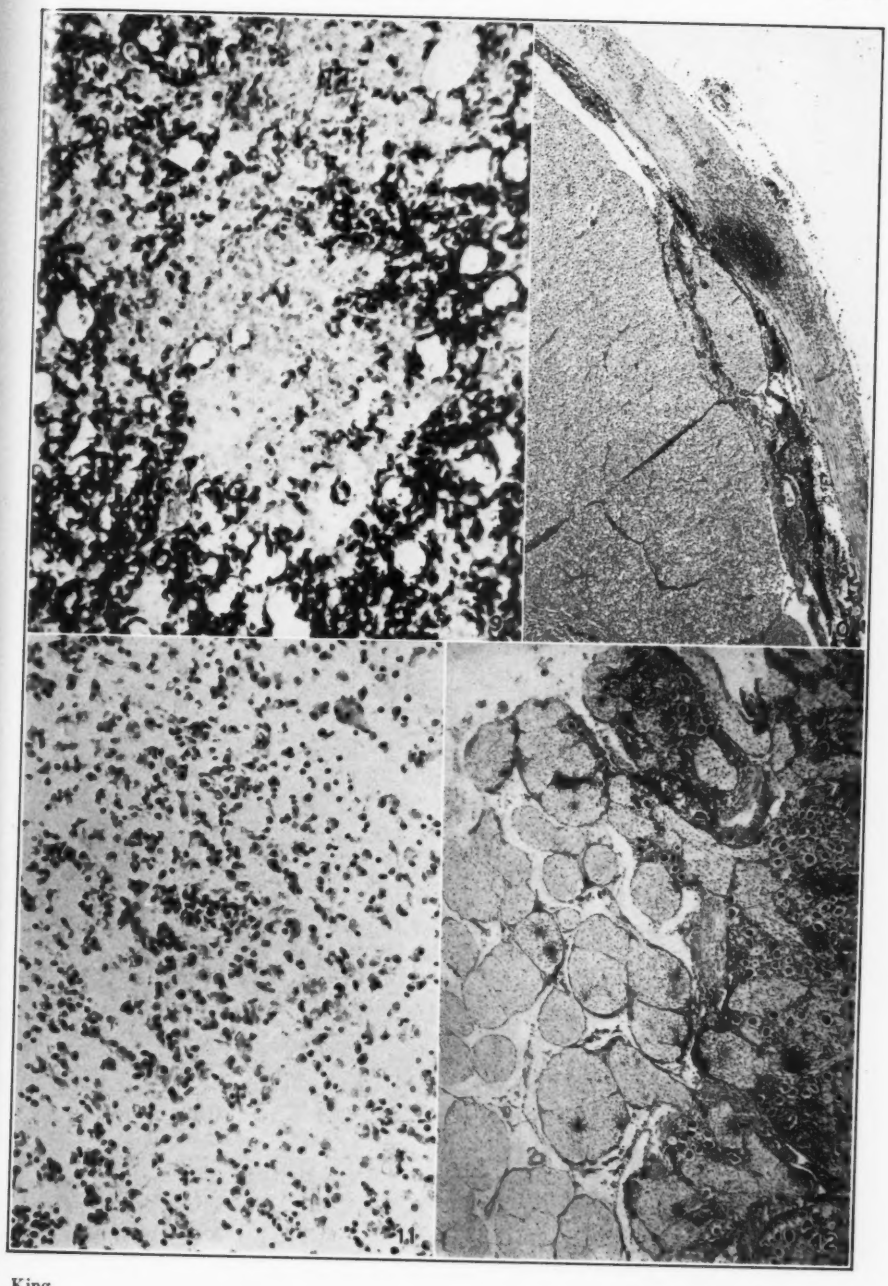
PLATE 101

FIG. 9. A lesion in the white matter of the spinal cord, stained for myelin. The pale area, corresponding to the focal collection of cells, contains many fragmented myelin sheaths and myelin droplets. × 292.

FIG. 10. Intense cellular focus in the dura overlying the spinal cord. At the lower right there is intense meningitis, but no focal myelitis in the subjacent parenchyma. Hematoxylin-eosin stain. × 33.

FIG. 11. Diffuse tissue infiltration, predominantly mononuclear, in the medulla. There is a severely injured neurone in the upper right corner. Toluidine blue stain. × 240.

FIG. 12. Inflammation of the gasserian ganglion and fifth nerve fibers. The focal collections of cells in the nerve bundles on the left are entirely similar to those seen in Figures 5 and 6. Toluidine blue stain. × 33.



King

Encephalomyelitis in Goats

SOLITARY GRANULOMA OF BONE

SIMULATING PRIMARY NEOPLASM *

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In 1932 a puzzling bone lesion was examined in our laboratory. Clinically it simulated a primary neoplasm of the rib, but it could not be classified with any of the usual types of bone tumor. The specimen was considered by Dr. Paul Klemperer to be a granuloma and was grouped by him during subsequent years with additional cases which appeared from time to time. The uncertainty as to the proper classification of this granuloma is evidenced by the fact that in 1 of these cases the possibility of early lipoid granuloma (Hand-Schüller-Christian's disease) was seriously considered. In all, we have seen 7 specimens of this lesion with identical histological features. Four of these are described in detail in this report, and only brief reference will be made to the remaining 3. In 1938 Schairer [1] reported 2 similar cases, but we have not found any other clear-cut reference to this disease.

CASE REPORTS

CASE 1.† R. B. (Mount Sinai Hospital No. P–16741), a boy aged 11 years, was admitted Sept. 11, 1939, and discharged Oct. 9, 1939.

The past history was negative except for lobar pneumonia 2 years previously.

While at camp during the summer 2 months before admission the patient complained of pain over the anterior right lower region of the chest. He had no recollection of injury in this area. Three weeks later a physician was consulted and X-ray films were made which showed a large defect in the right sixth rib (Fig. 1). There was no loss of weight or cough. The temperature was slightly elevated; the highest reached was 101° F.

On Sept. 9, 1939, the patient received a dose of preoperative radiation therapy: 200 r units to the mass, 140 kv., distance 50 cm. filter ½ mm. of copper, 3 mm. of aluminum, field 10 cm. by 10 cm. On Sept. 10, 1939, this dose was repeated and the patient was admitted to the hospital the next day.

Physical Examination: The patient was a healthy looking boy, normally developed. The nose and throat appeared normal. There were no enlarged

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† For the clinical data and use of the material in this case we are indebted to Dr. Harold Neuhof, Mount Sinai Hospital, New York City.

superficial lymph nodes. The liver and spleen could not be palpated. Physical examination of the heart was negative. Local examination revealed a definite swelling over the anterior right lower region of the chest with definite tenderness over the sixth rib. No definite mass could be made out but there was a sense of fullness to palpation. Auscultation was negative and no friction rub could be heard.

Laboratory Findings: Blood examination, the Wassermann test and a tuberculin patch test were negative.

Operation: A preoperative diagnosis of sarcoma of the rib was made. On Sept. 11, 1939, the day of admission, a partial excision of the chest wall was made. A liberal incision in the direction of the sixth rib was made anteriorly. The pectoral muscles were preserved as a flap. Along the sixth rib in the region of the midclavicular line there was a tumefaction. A specimen was removed for frozen section diagnosis, which was reported as a granuloma. The tumor was soft and succulent, and was limited to the sixth rib. The pleura was entered between the sixth and seventh ribs and the tumor was seen projecting into the pleura. A section of the sixth rib with the tumor and underlying pleura was excised (Fig. 2). The remainder of the pleura appeared normal, as did also the underlying lung. The muscular flap of pectoral muscle was mobilized and used to close the defect in the pleura and chest wall. This defect measured about 7.5 by 10 cm. The musculature as a freed flap was sutured to the soft parts and pericostal tissues at the lower rim of the defect. The skin and subcutaneous tissues were brought together without tension; no drainage was employed. A pneumothorax reading was done while the patient was still on the table.

Postoperative Course: The wound healed by primary union. There followed a prolonged series of febrile episodes, probably due to pneumonitis in the partially collapsed right lung. A pleural effusion developed but never became infected. At the time of discharge the patient's condition was fair, although he appeared somewhat under-nourished.

On Dec. 5, 1939, the patient was symptom free. He is now being treated by radiotherapy. He has regained his preoperative weight but there is still some fluid in the pleural cavity. The lung is expanding and the breath sounds come through.

Gross Specimen: The specimen consisted of a resected rib (Fig. 2) which measured 8 by 3 by 3 cm. The greater part of the specimen was occupied by a fusiform swelling, but there was a portion of normal rib 2 cm. long on one side, and of costal cartilage 1 cm. long on the other. The specimen was covered by parietal pleura on the inner aspect, while the remaining surfaces were covered by intercostal fat, fascia and muscle. There was a defect over the dome of the lesion where tissue for biopsy was removed. On longitudinal section there appeared an expanding lesion which thinned out and almost completely replaced the marrow space and cortex. The periosteum, however, could still be recognized (Fig. 2). The fusiform mass was grayish white in color with a yellowish

tinge. It was solid, but soft and cellular, without gross evidence of necrosis. The surrounding intercostal tissues appeared thickened, as though involved by the same process, and two millet-seed sized nodules were observed in the pleural covering. The transition to normal rib appeared gradual.

Microscopic Examination: A section (Fig. 3) taken through the entire specimen reveals a circumscribed lesion which is sharply defined from a normal costochondral junction on one side and which shades off into normal rib bone on the other. The lesion itself has produced a fusiform expansion of the cortex and periosseous tissues and consists of a granuloma with marked destruction and replacement of the osseous elements. Wide areas of the lesion show a complete absence of bone. The periosteum is considerably thickened and the process extends into the muscle adjacent and connective tissue (Fig. 7). This granuloma has destroyed not only the spongy bone of the marrow but also the compact bone of the cortex, and its shell or capsule is composed of periosteum and a new layer of callus bone on the inner aspect of the periosteum. The process fades out into a fibrosis of the marrow of the adjacent normal bone. The capsular demarcation is even less distinct histologically, for granulomatous foci can be seen at many points penetrating through the capsule into the surrounding muscle and fascia. The following are the main histological components of the granuloma: (1) large pale cells, usually in reticular arrangement; (2) leukocytes, mainly neutrophils, but also many lymphocytes and eosinophils *; (3) multinucleated giant cells, mainly of the osteoclastic type; (4) products of destruction — necrotic débris, broken-down fragments of bone, blood and blood pigment, lipophages; and (5) products of repair — fibrous tissue and callus bone.

(1) *Large Pale Cells:* These are present in great numbers in all portions of the lesion, alone or admixed with leukocytes and giant cells, outside the capsule as well as inside the lesion, in broad, ill-defined masses and in small circumscribed foci. They are comparable in structure and arrangement to the sinus endothelial cells of lymph nodes, *i.e.* large polygonal cells with an abundant pale cytoplasm whose processes interconnect with those of neigh-

* The eosinophils were not present in predominant numbers in this case, as they were in all the others.

boring cells (Fig. 6). There is a single oval nucleus which is also large and pale. The nuclear membrane is thin and often infolded, and the intranuclear chromatin substance is arranged in evenly distributed delicate strands. One or more tiny nucleoli (or chromatin clumps) are usually present also. Mitotic figures are not infrequent. In some areas these cells appear as free rounded elements (Fig. 5). Sometimes these cells store blood pigment and occasionally the cytoplasm has a foamy appearance associated with some lipoid infiltration which, however, is not doubly refractile. The conclusion was reached that these cells are histiocytes.

(2) *Leukocytes:* In this case, while several foci of eosinophils are present, the predominant leukocytes are neutrophils and lymphocytes, whereas in the other cases observed in this series, the eosinophils have predominated. The leukocytes are found in all portions of the lesion, including the extracapsular extensions. They are scattered lightly in some areas; in others they form compact masses. Some of the small nodular foci of large pale cells are surrounded by a compact zone of lymphocytes (Fig. 4).

(3) *Multinucleated Giant Cells:* Giant cells are present in small numbers in this case, scattered in small groups in different portions of the section. None are found in the pericapsular extensions of the process. These cells are of varying size and shape, are not very large, and usually contain about 4 to 10 nuclei crowded into the central portion of the cells. In general, they resemble the osteoclastic giant cells which are present in large numbers along the margins of the bony trabeculae.

(4) *Products of Destruction:* Near the center of the lesion a large zone of necrosis is present (Fig. 8). This region contains granular débris, blood, serum and degenerated cells. Bacterial stains in this area are negative, and cultures taken in the operating room from this specimen were sterile. Occasional foam cells and depositions of blood pigment can also be recognized. Here and there, in the midst of the granulomatous tissue, broken-down fragments of bone can be observed (Fig. 10). From their complete lamellar architecture these appear to be fragments of the original bone.

(5) *Products of Repair:* There is present a quite extensive deposit of callus bone in the capsule of the lesion (Figs. 3 and 7).

This is located on the inner aspect of the periosteum and extends for a short distance beyond the lesion over the surface of the neighboring normal bone. It does not form a complete layer but exhibits several gaps through which the granulomatous process extends into the surrounding tissues. The callus shows a quite complete transformation into bone and its main trabeculae are laid down in radial fashion. Areas of fibrosis are present within the granuloma as well as in some of the pericapsular foci. The cellular portions of the lesion are permeated by capillaries with conspicuous endothelial cells, as commonly found in granulation tissue.

Comment: In general, this lesion appears as a granulomatous, not neoplastic, process in the rib. It is fairly well circumscribed, although it extends also into the surrounding muscle and connective tissue. It is associated with extensive destruction of the preëxisting bone, and there is some bone replacement by means of callus formation from the periosteum.

CASE 2.* R. R., a boy, aged 7 years, was admitted to the Lebanon Hospital on Oct. 28, 1937, and discharged Dec. 5, 1937.

The family and past history were unimportant.

Two weeks ago the mother attempted to lift the child from the floor by pulling his right arm, which was twisted during this act. The child subsequently complained of pain in the right shoulder and inability to move the arm. The pain subsided somewhat and the child was taken to the out-patient department of the hospital. Clinical and X-ray examinations were suggestive of tuberculosis or malignant neoplasm, and the child was admitted.

A roentgen film (Fig. 12) report on Oct. 25, 1937, read as follows: "Radiographic examination of the right shoulder shows an area of bone destruction, fairly extensive, involving the glenoid fossa and the neck of the scapula, and extending along the outer border. There are some productive changes in this region. The upper end of the humerus and the epiphysis of the head show no definite changes. The lesion can be due either to neoplasm involving the scapula or to chronic osteomyelitis. It is impossible to determine at this time the exact nature of this lesion involving the scapula. Examination of the left shoulder shows no abnormalities."

Physical Examination: This showed a pale child of normal build. The general examination was negative. The right shoulder was fuller than the left and there was tenderness on motion of the right shoulder in all directions. The other extremities were normal.

On Nov. 1, 1937, another X-ray of the right shoulder was reported as follows: "Radiographic examination of the right shoulder discloses a slight increase in the productive changes. Otherwise the lesion appears approxi-

* For the clinical data and use of the material in this case we are indebted to Dr. Louis Sheinman, Lebanon Hospital, New York City.

mately the same. Examination of the chest fails to show any abnormality in the lungs. The heart is normal in size, shape and position. Examination of the left shoulder fails to show any abnormality. At this time, we still feel that the lesion may be due either to neoplasm or to chronic osteomyelitis. We are more inclined to favor the first conclusion. We would advise re-examination in about 10 days for further study, as it is impossible at this time to make a definite conclusion."

Laboratory Findings: Urine and blood examinations were normal. The Wassermann test was negative. Chemical examination of the blood was normal. The phosphorus was 5 mg. per cent, calcium 10.4 mg. per cent, and the phosphatase 6 units (Bodansky). Repeated phosphorus and calcium determinations gave similar figures. On Nov. 16, 1937, the phosphatase was 4 units and on Nov. 17, 1937, 3.5 units. The Mantoux test was negative on three occasions.

The temperature ranged between 99.2 and 100.6° F. The pulse was 90 and the respirations 20 to 25.

Operation: On Nov. 10, 1937, operation for biopsy and removal of material for histological diagnosis was performed. An incision 13 cm. in length through the muscles of the scapula was made. The periosteum was elevated and about 2 cc. of the soft material from the tumefaction was curetted away. A culture was taken and the cavity was packed with vaseline gauze. Smear and culture did not reveal any bacteria. Another roentgen film (Fig. 13) made on Nov. 26, 1937, was reported as follows: "Radiographic examination of the right shoulder in comparison with previous film now discloses considerable bone production in the region of the glenoid fossa and along the lateral border of the scapula."

Postoperative Progress: The wound healed rapidly and closed. The patient's condition remained excellent at all times and he was discharged on Dec. 5, 1937.

Microscopic Examination: Sections reveal two types of tissue. One of these consists of the cortical and spongy bone. This tissue appears quite compact but is otherwise relatively normal. The haversian canals and marrow spaces are filled with fibrous fatty marrow. Occasional osteoblasts and osteoclasts are present. The cement lines do not show any unusual disturbances. The lacunae and bone cells appear essentially normal.

The second type of tissue is represented by rather large and very cellular masses of tissue which do not contain any bone trabeculae. There are four main types of cells present in this tissue. These cells consist of (1) large numbers of typical giant cells of the osteoclastic type (Fig. 14). (2) Enormous numbers of eosinophilic polymorphonuclear cells (Figs. 14 and 15). (3) Large mononuclear pale cells, either free and round or in a reticular arrangement (Figs. 14 and 15), and which have a round or folded nucleus. Occasional cells are larger and contain two or three nuclei. The cells bear a general resemblance to macrophages.

(4) Collections of lymphocytes. There are a few small areas of necrosis present in the tissue.

Comment: This histological picture does not conform to any known type of bone tumor. However, identical histological pictures were encountered in my experience once before; at Memorial Hospital once or twice, and at Mount Sinai Hospital several times. F. C. (Case 3) is today alive and well without recurrence of a new disease area after 2 years. A similar excellent prognosis is indicated in the other cases described above. F. C. (Case 3) received postoperative radiation but no radical surgery.

The specimen was diagnosed as an unclassified granulomatous lesion. Postoperative radiation, but no radical surgery, was recommended. The local lesion healed (Fig. 13) with much new bone production, and the patient is alive and well at the time of writing.

CASE 3. F. C. (No. E 317), a girl, aged 15 years. The only data available is that this young girl had noted a swelling over one rib for several weeks. At operation a fusiform, egg-shaped swelling of the rib 3 by 5 cm. was found. Portions were removed and about 4 cc. of tiny irregular fragments of tissue were sent to the laboratory for diagnosis.

Microscopic Examination: The appearance of this tissue in paraffin section is characterized by a very cellular growth composed of large pale cells whose appearance is quite uniform in all fragments examined. These cells have a large infolded vesicular nucleus and an abundant pale eosinophilic cytoplasm which is irregularly outlined and appears in the form of a network, so that the cytoplasmic processes of the various cells interlace with one another. Mitotic figures appear quite frequently in the nuclei of these cells, but none of the mitoses are atypical. This network of pale cells is everywhere infiltrated by enormous numbers of eosinophilic leukocytes which in some areas appear as broad sheets and cords of cells. Also, occasional giant cells are present among the pale cells described above. These contain four to eight nuclei, are fairly round in outline and have an abundant amount of cytoplasm. This cellular appearance, the mitoses, the giant cells and the eosinophilic leukocytes, are the characteristic features of every fragment of tissue sectioned. There are no bone particles present. There are many small areas of hemorrhage.

Comment: This tumor should not be classified with any of the

common types of bone tumor. The possibility of an immature Ewing's tumor must be considered. There is also a possibility, from comparison with similar slides, of a granuloma, in which case the prognosis would be much better. Because of the cellularity and mitoses radiation therapy was recommended.

This patient is alive and well at the time of writing, there is no recurrence, and the local lesion is healed.

CASE 4.* L. (No. P–5229), aged 35 years, was admitted to the Mount Sinai Hospital on Jan. 7, 1932, and discharged 14 days later. No data on the history were available.

The patient was operated upon Jan. 8, 1932, and the pathological report was as follows:

Gross Examination: The specimen consisted of a resected rib 8 cm. in length. In approximately the midportion there was a fracture. At the site of the fracture there was a nodular thickening of the bone. Its architecture appeared disorganized and in the central portion the normal marrow cavity was replaced by pale gray cellular tissue.

Microscopic Examination: Sections show the bone marrow cavity to be expanded and filled by a very cellular tissue which encloses a few fragments of bone trabeculae, probably the remnants of the spongy bone. The tissue consists of large polygonal cells with pale, oval shaped, occasionally infolded nuclei, a large number of eosinophilic leukocytes and lymphocytes. There are small areas of necrosis. The periosteum is strikingly thickened and there is formation of young bone on its inner surface. No giant cells are present. A diagnosis of inflammatory bone lesion with fracture of the rib was made.

DISCUSSION

A comparison of the findings in these 4 cases, and in 3 other cases which we had the opportunity to examine, enables us to give a general description of this lesion. Solitary granuloma of bone occurs in young individuals or children and appears clinically as a single, painful, tender and swollen lesion in a bone of several weeks or months duration. There may be a history of local injury to the area affected. Physical examination is generally negative

* For use of the material in the case we are indebted to Dr. Howard Lilienthal, Mount Sinai Hospital, New York City.

except for the local lesion, and the usual laboratory tests yield normal results. Roentgenograms disclose an expanding and destructive lesion with irregular new bone formation extending into the soft tissues. At operation a soft encapsulated tumefaction is found which can easily be curetted. Cultures and smears are negative. Histological examination of the tissue has disclosed an essentially identical appearance in all cases without exception. The sections reveal unmistakable evidences of a granulomatous process which is non-specific in character. Recurrences or additional lesions in other areas have not occurred and the general health remains unimpaired. Gradual healing of the local lesion appears to take place. In the longest case followed, the patient is alive and well after 5 years. Various bones may be involved, for our series includes cases in the rib, skull, sternum and mastoid region.

There is no resemblance to Hodgkin's disease or to specific infections. Furthermore, the various well known primary bone tumors are not suggested in the differential diagnosis, although the possibility of an atypical Ewing's tumor may come up for consideration. The absence of plasma cells and of conspicuous fibrosis, and the negative bacteriology, differentiate this lesion from the usual forms of chronic osteomyelitis.[2, 3] Aside from the giant cells, which appear to be reactive in origin, this lesion bears no resemblance to giant cell tumor. Furthermore, in 1 of our cases no giant cells were present (Case 4). The presence of lipophages might raise the question of an isolated lipogranuloma (Hand-Schüller-Christian's disease). In those cases in which examination for doubly refractile fat was made, none was found. This would appear to rule out the diagnosis of lipogranuloma, and the further course of these cases with spontaneous healing and without any evidence of generalization would seem to separate this granuloma from the category of Hand-Schüller-Christian's disease.

The opinion that this disease is granulomatous is based upon the histological findings, especially of Case 1, where it was possible to examine the entire lesion. Here, numerous sections examined from all portions failed to reveal any underlying neoplastic process. The predominating cellular elements which constitute the lesion are those which commonly make up granulomas. Furthermore, the stroma, with numerous capillaries lined by

conspicuous endothelium, is of the type commonly associated with granulation tissue.

Sections from the remaining 3 cases reported in this series, and also from 3 additional cases which we had the opportunity to examine, have presented essentially similar if not identical histological features. In 1 case (Case 1), while foci of eosinophils were present, neutrophilic leukocytes predominated. In another case (Case 4) no giant cells were seen. In Case 1 a relatively large area of necrosis was present, but this may have been related to the two doses of X-ray therapy which were administered before operation. Aside from minor variations of this type, the histological picture in all cases was practically identical, to such a degree that the opinion is ventured that one is justified in rendering a diagnosis of "solitary granuloma" from the histological examination of a bone lesion alone.

The occurrence of a histological complex as above described raises the question of etiology. Schairer [1] favors trauma as the initiating factor, basing this opinion on a consideration of the histories and histological and X-ray appearances in his own 2, as well as in several reported cases, which he thinks belong to this group. In our own series several factors appear to point to trauma as initiating the granulomatous process. In Case 1 no history of trauma could be obtained. In Case 2 there was a history of trauma several weeks before the child was admitted to the hospital. There were no records of the history in Cases 3 and 4. However, in Case 4 the surgical specimen presented a definite fracture. In Case 5 there was a definite history of trauma. This patient stated that he had been struck on the head by a baseball in the exact location where the granuloma subsequently developed. A similar clear history of trauma to the site where the lesion developed was available in Case 7. In fact, in this case two lesions existed and there was a history of separate trauma to each area where these lesions developed.

In general, however, the establishment of a causal relationship between the injury and lesion on the basis of history, particularly where young individuals or children are questioned, is obviously difficult. Schairer gives the possibility of initial trauma additional weight because the lesion is most common at an age when traumas are frequent.

We find in such considerations one suggestion that trauma may

initiate this lesion. Some of the anatomical features also point to trauma as an etiological factor. Examination of the sections of the specimen in Case 1 reveals the existence of a dissolution of continuity over a broad zone (Fig. 3). The gap is covered by periosteum, on the inner surface of which a recent layer of callus bone has been laid down (Figs. 3 and 7). Within the granuloma, broken-down fragments of the original bone may be recognized here and there (Fig. 10). Furthermore, an examination of the periosteum reveals that it has been dissected and frayed out in a manner which can best be explained by trauma with a fracture.

If trauma is actually the initiating factor in this disease, another and perhaps more difficult problem is raised, namely the peculiar and unusual form which the response to this trauma assumes. The large pale cells and accumulations of eosinophils (Fig. 15) which distinguish the histological picture are not the usual responses to bone injury. On the other hand, similar cellular reactions may be observed in other tissues, usually in connection with infection. Several of our cases have had careful bacteriological cultures made with completely negative results. Schairer also reports negative bacteriological findings. We do not believe that the peculiarity of the response is related to any specific granulomatous process such as Hodgkin's disease or to any specific infection. It appears that some as yet unknown factor is responsible for the unusual form which this process assumes.

The sections from Case 1 indicate that the process in the involved area is chronic with repeated exacerbations. The older areas can be recognized because already the granulation tissue has undergone some fibrosis. The younger areas, on the other hand, appear at many points where the histological picture is dominated by nests of young histiocytic elements. In a few areas, small nests of these histiocytes appear to have just formed, and to push aside the already existing granulation tissue and dense collections of lymphocytes. However, the healing process is already in evidence in the form of fibrosis and callus bone formation. In Case 2 the extensive lesion illustrated in the first X-ray film (Fig. 12) is seen to have disappeared almost completely in the second X-ray film (Fig. 13) after an interval of 9 months, during which radiotherapy was administered. In this case most of this granuloma was left behind at operation, since only a small portion

was removed for histological diagnosis. Similar healing is known to have occurred without radiotherapy in Case 5.

As a result of our observations we do not feel that postoperative radiotherapy for the repression of malignant tendencies is justified. The possibility of its use to stimulate healing or to eliminate chronically exacerbating foci within the lesion may be considered.

Summary and Conclusions

1. A group of cases of a solitary bone lesion in young individuals with clinical and roentgenological features suggesting a primary neoplasm of bone is reported. Histological study revealed a granuloma in all cases.

2. The histological picture was identical in all instances and was characterized by cellular tissue composed mainly of histiocytes and eosinophils.

3. The clinical course indicates that the lesion is benign.

4. The etiology is unknown, but our material indicates that trauma may play some rôle in initiating this lesion.

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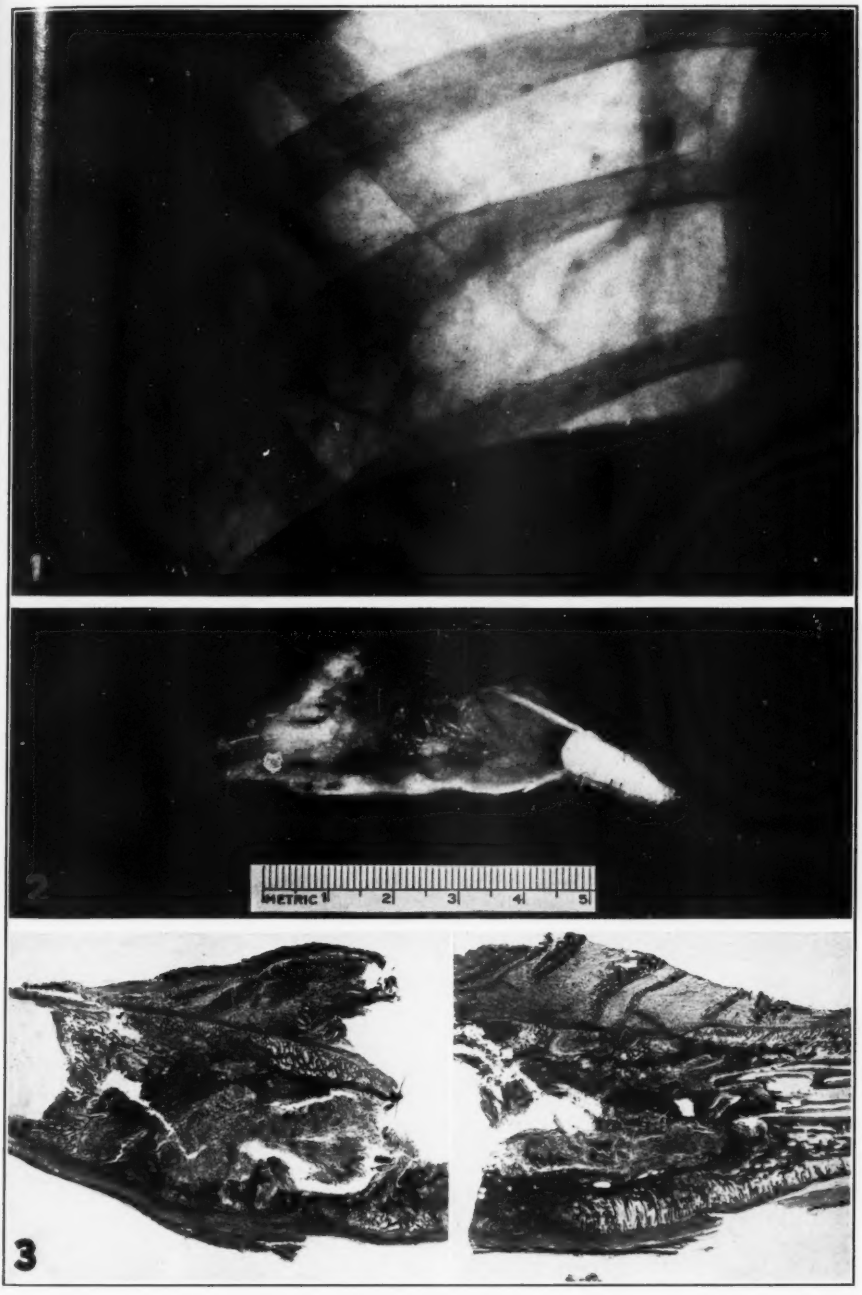
DESCRIPTION OF PLATES

Plate 102

Fig. 1. Roentgen film of lesion in the right sixth rib in Case 1.

Fig. 2. Longitudinal section of surgical specimen from Case 1. The defect on the upper surface was produced during removal of the specimen for frozen section diagnosis. Note the homogeneous cellular character of the lesion. Cartilage is seen on the right.

Fig. 3. Low power microphotograph of right and left halves of the specimen shown in Figure 2. Cartilage is seen on the left, normal rib bone on the right. Involvement of the periosseous tissues may be noted above. Also, newly formed bone can be observed on the inner aspect of the periosteum. Note that this callus bone extends over the normal rib cortex at the right. × 2.5.



Otani and Ehrlich Solitary Granuloma of Bone

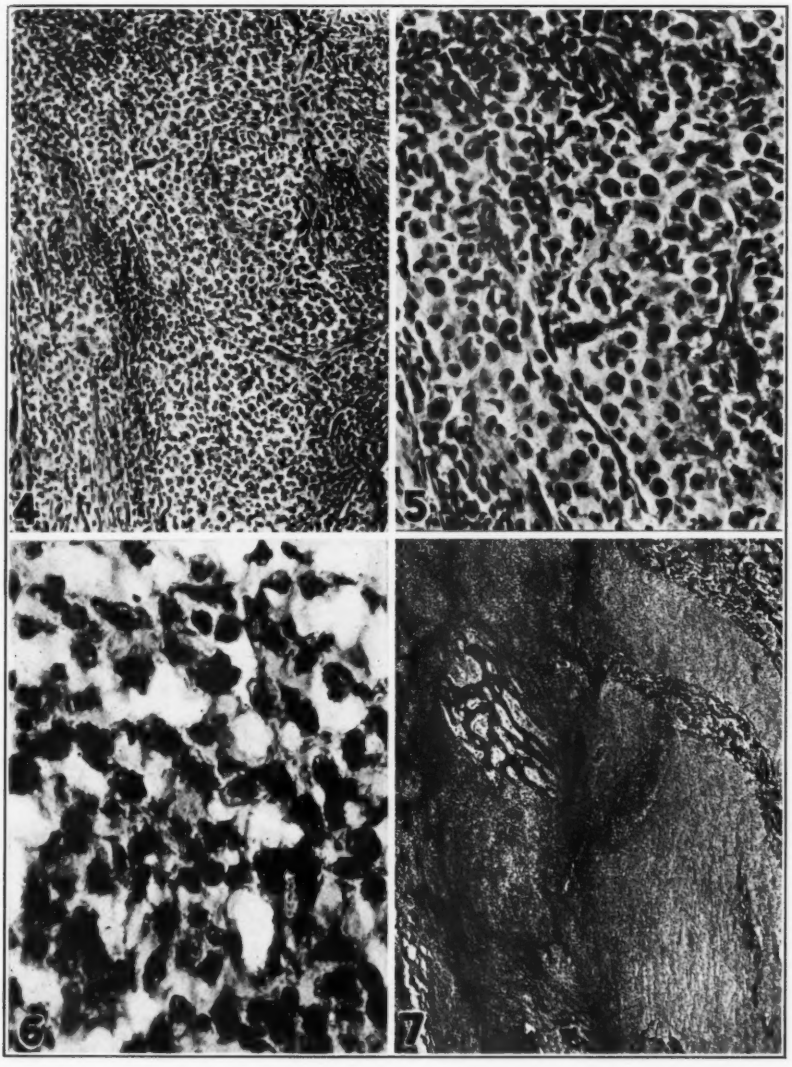
PLATE 103

FIG. 4. Low power view of the typical appearance of granuloma showing nests of histiocytes and collections of lymphocytes. × 60.

FIG. 5. Same field as in Figure 4, higher magnification. × 200.

FIG. 6. A group of histiocytic cells showing reticular arrangement. × 400.

FIG. 7. Low power view of the periphery of the lesion showing muscle and connective tissue to the right and granuloma to the left. Note the island of callus bone and small granulomatous lesion outside the periosteal region. × 12.



Otani and Ehrlich Solitary Granuloma of Bone

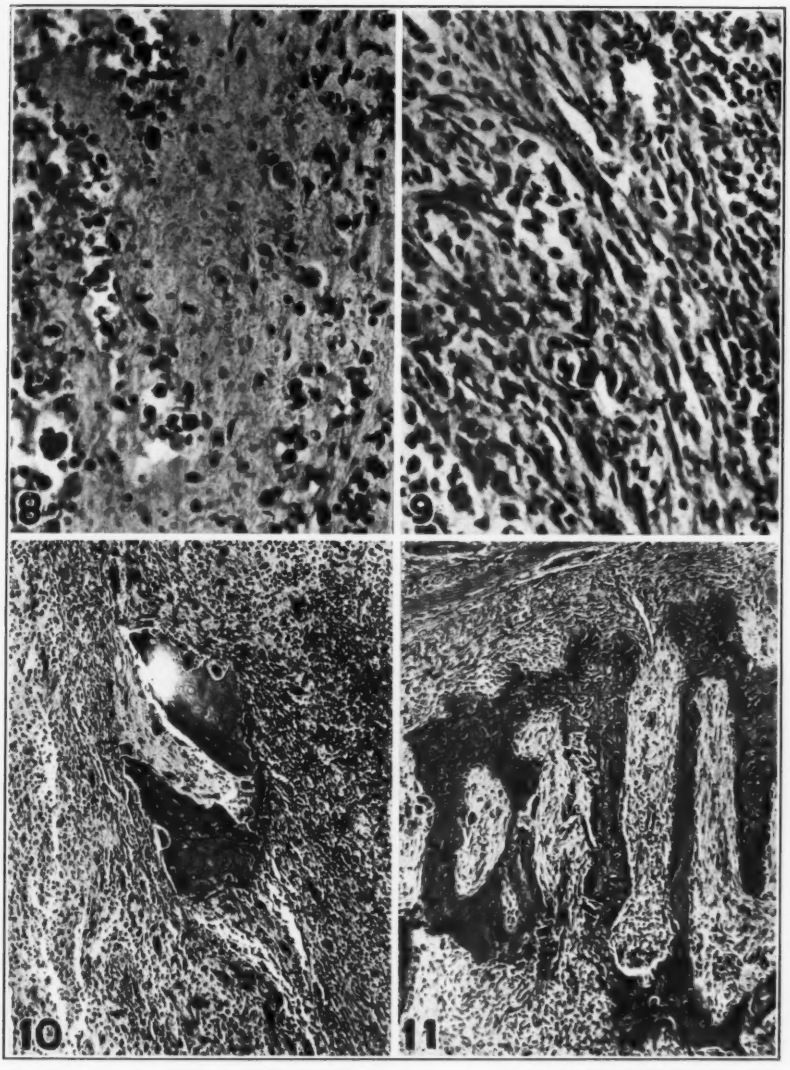
Plate 104

Fig. 8. Area of necrosis. × 200.

Fig. 9. Area of granulation tissue undergoing fibrosis. × 200.

Fig. 10. Fragment of original bone encased in granulation tissue. × 60.

Fig. 11. Newly formed bone on the inner aspect of the periosteum. Periosteum above. × 40.



Otani and Ehrlich Solitary Granuloma of Bone

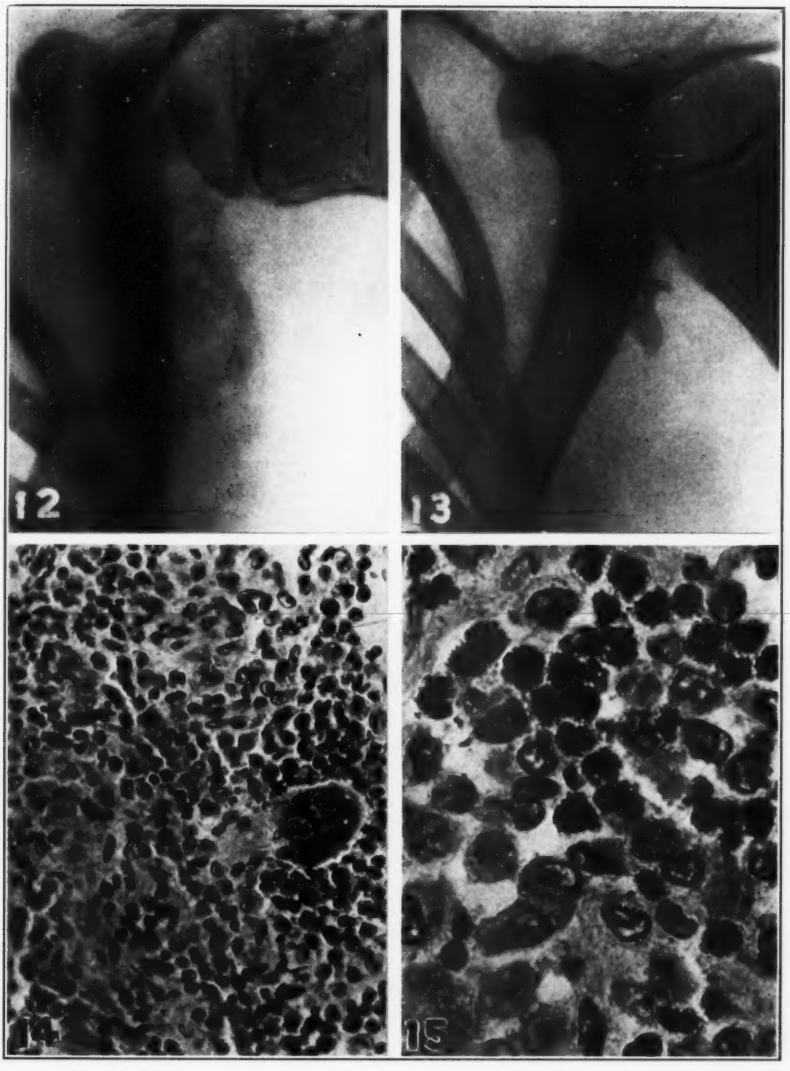
PLATE 105

FIG. 12. Roentgen film of scapula lesion in Case 2 before operation.

FIG. 13. Roentgen film of scapula lesion in Case 2, 9 months after biopsy and postoperative radiotherapy.

FIG. 14. Characteristic field showing histiocytes, eosinophils and giant cell in Case 2. × 200.

FIG. 15. Same field as Figure 14. High power view of histiocytes in reticular arrangement and eosinophils (black cytoplasmic granules). × 700.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EFFECTS OF OVARIECTOMY AND LONG CONTINUED ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT OF CATTLE ON SKELETAL TISSUES OF IMMATURE GUINEA PIGS *

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In a previous short communication [1] we reported that in growing guinea pigs, in the first 4 weeks following ovariectomy, the proliferation of the epiphyseal cartilage is increased and simultaneous injections of anterior pituitary extract of cattle promote calcification and ossification in the epiphyseal disk. In continuation of these investigations we studied more fully the changes which occur in various cartilaginous and bony tissues following ovariectomy, in particular those occurring at later stages. These additional experiments seemed to be of interest also because our previous observations did not allow any conclusions as to the permanence of the changes produced by ovariectomy. Furthermore, we wished to determine whether the continuance of the effects of ovariectomy would be interfered with by prolonged administration of anterior pituitary extract. Finally, we wanted to compare the reactions of the skeletal tissues under these experimental conditions with the normal processes of maturation of cartilage and bone.

MATERIAL AND METHODS

Twenty immature female guinea pigs, each weighing approximately 140 gm., were ovariectomized. Eleven of them were subjected to ovariectomy without receiving injections of the anterior pituitary extract, while 9 ovariectomized animals received daily intraperitoneal injections of 1 cc. of acid extract of anterior pituitary of cattle. In those animals which were greatly weakened and which lost weight rapidly during the first 2 weeks of the injections,

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the dose was temporarily reduced to 0.5 cc., or, in some instances, the injections were discontinued for 1 or 2 days. Of 11 ovariectomized guinea pigs not receiving injections, 2 were examined after 6 weeks, and 2, 3 and 4 months, respectively, and 1 animal after 8, 9 and 17½ months. Of the 9 ovariectomized and injected guinea pigs, 1 was examined after 5 weeks, 2 after 6 weeks, 2 after 2 months, 3 after 3 months, and 1 after 4 months.

The tibiae, femurs, ribs and vertebrae were removed for microscopic examination and were subjected to our usual technic of embedding and staining.[2]

Observations

In comparison with normal animals the ovariectomized guinea pigs gained weight more quickly within the first 2 months. Later, an increase in weight occurred likewise, but it took place more slowly. In contradistinction to the animals which were merely ovariectomized, the ovariectomized guinea pigs which were injected with pituitary extract either did not gain or they even lost weight within the first 2 weeks, their weights falling sometimes below the initial figures. Between 2 and 6 weeks following the beginning of the injections the weights rose again slowly, but still remained decidedly about 40 per cent lower than those of the corresponding ovariectomized animals. After 2 months of injections of the extract their weight began to equal that of the non-injected ovariectomized animals, and after 3 and 4 months no marked difference existed between the weights of these two sets of animals. The weights of the animals are given in Table I.

Grossly, the long bones of the ovariectomized animals appeared more slender than normal ones. On frontal as well as on sagittal sections through the decalcified tibiae, the upper epiphyseal line was always distinctly visible as a translucent, continuous whitish stripe, even as late as 17½ months following ovariectomy; whereas, under normal conditions, at this age the epiphyseal line had completely disappeared or was indicated only by transverse trabecular osseous structures. The ribs were thin and flat. Conversely, the long bones and the ribs of the guinea pigs which had been treated with the pituitary extract subsequent to ovariectomy were harder and thicker than those of the guinea pigs which had only been ovariectomized.

TABLE I

Mean Weights and Deviations from the Means

	Initial weights	Weights after								
		4 wks.	5–6 wks.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	12 mos.	17½ mos.
Ovariectomized guinea pigs	(11 Gp's) 144+11 − 9	(11) 270+35 −45	(11) 311+49 −51	(9) 364+51 −54	(7) 444+66 −74	(5) 511+64 −126	(3) 630+100 +125	(3) 648+102 −133	(1) 790 ...	(1) 890 ...
Ovariectomized and injected guinea pigs	(9 Gp's) 149+6 −4	(9) 193+37 −38	(9) 242+73 −77	(6) 357+53 −92	(3) 460+40 −45	(1) 565 ...	... ...	... ...	... ...	... ...

Microscopic Examination

I. Normal Guinea Pigs:

We have reported previously [3] on the condition of epiphyseal cartilage and bone in mature and immature guinea pigs. The first signs of beginning osseous closure of the epiphyseal zone of the upper tibia were not seen before the animals had reached a weight of 400 gm., and an age of about 3 to 4 months. In guinea pigs weighing 800 gm. and about 8 to 12 months old, the proliferation of the cartilage had almost ceased, and the union of metaphysis and epiphysis was progressing. It was completed in animals which were in the 2nd year of life.

II. Ovariectomized Guinea Pigs:

Epiphyseal line: The zone of endochondral ossification was enlarged during the first 4 months following ovariectomy. It was only 8 to 9 months after ovariectomy that the epiphyseal line became narrower, although it was still relatively wide as compared with that of the normal controls of the same age. Even as late as 17½ months following ovariectomy the epiphyseal disk, although narrow, was still recognizable as such and consisted of remnants of euhyaline cartilage.

During the first 3 months the cartilaginous matrix was diminished in amount, slightly swollen and disintegrated, and comparatively little calcium was deposited at the periphery of the hypertrophic cartilage cells. The amount of matrix and calcium had increased after 4 months. After 8 months the cartilaginous ground substance became fibrillar, denser, sclerosed and converted into preosseous material.

During the 1st months the resting cartilage cells were more numerous and larger than normally. The columnar cartilage cells were flat and had undergone a pronounced hyperplasia (Fig. 1). One single row of cartilage cells could contain as many as from 15 to 18 cells, as compared with a normal count of 10 cells; here and there a mitotic division occurred, but as a rule proliferation took place by way of amitosis. Only after 4 months, owing to an increased sclerosis of the cartilaginous matrix, and a shrinkage and disintegration of some cartilage cells, the rows of cartilage cells became less numerous. After 8 months (Fig. 2) the greater part of the epiphyseal line still consisted of cartilaginous material,

which was, however, poor in cells. Even 17½ months after ovariectomy, although the main mass of the cartilage had undergone increasing ossification, rudimentary columns of cartilage cells were still visible (Fig. 3).

At all stages the layer of the hypertrophic cartilage was sharply demarcated from the bone marrow and its replacement by bone was, as a rule, regular. Only in 1 case, 2 months following ovariectomy, were strands of hypertrophic, calcified, but only incompletely ossified cartilage cells noted. These strands reached downward into the subepiphyseal zone. After 4 months the number of hypertrophic cartilage cells had somewhat decreased, the deposits of calcium at the periphery of these cells had become more marked, and ossification was somewhat intensified as compared with earlier periods. The very first signs of deposition of osseous substance in the epiphyseal line could be observed in 2 guinea pigs which had reached a weight of 500 and 560 gm., as evidenced by the appearance of isolated, oblong, delicate osseous plugs which traversed the zone of endochondral ossification in the direction from the subepiphyseal bony trabeculae to the epiphyseal bone. Normally at this stage osseous plugs and bony bridges were more numerous and thicker. With increasing duration of the experiment the hypertrophic cartilage cells became fewer, and after 17½ months no appreciable number of these cells could be detected. Simultaneously, the longitudinal bony plugs gradually increased in number and size.

Chondrophyte: The cellular constituents of the lateral protuberances reacted in a similar manner to the cells of the epiphyseal zone. With increasing hyperplasia of the epiphyseal cartilage the precartilaginous and the mature euhyaline cartilage cells of the chondrophyte became likewise more numerous and the intercellular matrix diminished. In the most characteristic cases the euhyaline cartilage cells showed amitotic divisions or they coalesced in such a way that, in some instances, four or an even larger number of enlarged single cells were surrounded by a single capsule. Thus, hypertrophied incubator capsules were produced. After 4 months the signs of growth stimulation gradually receded and the cartilage became more of the resting type. After 8 months an almost inactive state was seen, such as is normally present.

Joints: Between 6 weeks and 2 months following ovariectomy the cells of the transitional and pressure zones were markedly hyperplastic and hypertrophic and they took on a perpendicular instead of the normal horizontal arrangement. Some underwent karyorrhexis and karyolysis and became liquefied, and small vacuoles took their place. The osseous border lamella, which ordinarily separates the bone marrow from the cartilage, and the layer of hypertrophic cartilage cells were in many instances corroded by capillaries advancing from the bone marrow. These vascular loops could penetrate far into the transitional and pressure zones. Owing to these resorptive processes, the cartilaginous covering became thinned out and only a little bony substance was laid down. After 3 and 4 months the conditions were essentially the same in kind, but the above changes in the cartilage had gradually diminished in degree. The covering of the joint appeared thinner but still contained many cells. Eight months following ovariectomy the cells of the articular cartilage had returned to an almost resting state, such as is seen under normal conditions. No severe arthropathic lesions were detected. However, the amount of bone deposited was less than normal.

Marrow Cavity: During the first 2 months the capillaries were congested. In the subepiphyseal layer a slight fibrosis of the marrow was produced in some instances. But after 3 months and later, an almost normal behavior was again seen. The osseous spiculae were fairly numerous, slender, thin and elongated, a condition which became particularly noticeable in the very late stages (Fig. 3). The trabeculae were surrounded by many epithelioid cells showing mitotic figures. Some of these cells arranged in a bead-like manner acted as osteoblasts penetrating into and dissolving the bony substance; others coalesced, formed giant cells and acted as osteoclasts.

The Bony Shaft: During the first 2 months the periosteal connective tissue and its fibrils were loosened. The connective tissue cells of the periosteum proliferated freely by way of mitosis. In the 2nd month numerous epithelioid cells appeared along the bony compacta and invaded the osseous substance in several places. Thus, wider lacunar spaces and grooves were formed filled with multinuclear giant cells which absorbed the compact bone. The haversian canals were likewise enlarged and converted into wider

spaces containing congested capillaries, a condition which also caused an increased solution of the bone. Owing to this stimulation of resorptive processes the compacta of the bony shaft became somewhat thinner than it is ordinarily. Similar were the findings at later stages of the experiments, but at this time the fibrils of the periosteal connective tissue became gradually thickened and somewhat sclerosed. Still later, the surface of the compacta became smoother, the lacunar grooves disappeared, and the haversian canals were again narrower. However, the cortex remained slender and thin and it resembled in this respect the trabeculae of the marrow cavity.

The Ribs: In the cartilaginous portion the ground substance was slightly loosened and the cartilage cells underwent a slight hyperplasia in the early stage following ovariectomy, but after 3 months or later the matrix became increasingly sclerosed; however, calcification was decreased in comparison with the normal condition. The replacement of the cartilage by bone was delayed but otherwise not disturbed; the epiphyseal zone was regular and demarcated by a straight line from the marrow. The trabeculae and the bone marrow showed a picture similar to that seen in the tibia.

III. Ovariectomized Guinea Pigs which were Injected with Anterior Pituitary Extract:

Epiphyseal Line: This was narrower than in the corresponding stages of the non-injected ovariectomized guinea pigs and it contained fewer cells. The degree of narrowing varied considerably in different cases, as shown in Figures 4 and 5.

After 6 weeks the cartilaginous ground substance became thickened and sclerosed, resembling preosseous and osseous substances. In some cases larger amounts of calcium salts were deposited at the periphery of the cartilage cells. Between 2 and 4 months after ovariectomy the sclerosis and ossification were intensified, whereas the calcification gradually receded.

At early stages the resting cartilage cells assumed an oval shape; they became enlarged and proliferated. The columnar cartilage cells underwent retrogressive changes which predominated in the first 2 months. Atrophy, karyorrhexis and karyolysis were seen and cartilage cell rows were destroyed. Some cells, the capsules

of which had been dissolved, enlarged, rounded off, and took on an epithelioid shape. In accordance with the varying degrees of reactivity of the cartilage, a more or less irregular arrangement of the cartilage columns could result.

In some instances the hypertrophic cartilage cells were densely calcified (Fig. 5), and then their replacement by bone was incomplete, as indicated by the presence of calcified, non-ossified, hypertrophic cartilage cells in the subepiphyseal layer. As early as 6 weeks after the beginning of the injections degenerated areas were not infrequently found to be replaced by bony substance. Osseous plugs were formed in the epiphyseal disk, extending from the bony trabeculae of the metaphysis into the epiphysis (Fig. 4). The state of ossification at this stage was comparable to, but farther advanced than, that seen in ovariectomized non-injected animals 4 months subsequent to ovariectomy. After 3 and 4 months these osseous plugs were more numerous and thicker. The intensity of ossification was greater than in guinea pigs which had merely been ovariectomized, but less than in the injected animals with intact ovaries, and also less than in normal control animals.

Chondrophyte: The precartilage and cartilage cells were increased in number and size in the first 2 months of the experiments, the intercellular substance being diminished. In some places, particularly in those adjoining the epiphyseal disk proper, the proliferating and enlarged cartilage cells of the euhyaline type formed hypertrophic incubator capsules. After 3 and 4 months, however, the conditions returned more and more to the normal resting stage.

Joints: Between 6 weeks and 2 months the cells of the transitional and pressure zones were more numerous than ordinarily. The cells of the sliding zone proliferated moderately. Associated with these proliferative processes was an enlargement of the cells, which became more accentuated in the areas adjoining the hypertrophic cell layer. Subsequently, the cells underwent karyorrhexis, karyolysis and liquefaction. Capillary loops advanced from the bone marrow and corroded both the osseous border lamella and the hypertrophic cartilage cells. The former was thickened as compared with the lamellae in ovariectomized non-injected guinea pigs. After 3 and 4 months the proliferation and capillary corrosion gradually receded, whereas ossification was still progressing. The

cartilaginous ground substance became denser and resembled more and more preosseous and osseous substances. Owing to the previous proliferation of the cartilage cells and to the subsequent deposition of larger amounts of bone, the covering of the joint was thicker than in the ovariectomized guinea pigs which had not received injections of anterior pituitary extract.

Marrow Cavity: Six weeks to 2 months after ovariectomy and the beginning of the injections, the osseous layer separating marrow cavity and epiphyseal cartilage was in some instances irregular. In the subepiphyseal layer rows of partly calcified cartilage were seen, which were not at all or only incompletely ossified (Fig. 5). These strands of cartilage were surrounded by a connective tissue containing a variable amount of fibrils. Proliferating connective tissue cells became either converted into osteocytes or acted primarily as osteoclasts dissolving the bone and causing a thinning of the osseous spiculae. However, thick and regular bony trabeculae were also formed, which were surrounded by mitotically proliferating epithelioid cells. The latter changes were seen particularly at later stages, when the apposition of bone predominated over the processes of resorption. Thus the formation of bone was greatly increased as compared with conditions in ovariectomized guinea pigs not receiving injections of the extract.

The Bony Shaft: The periosteal cells proliferated and the production of epithelioid cells was increased in contact with the bone. As was the case in the bone marrow, they acted here also at first as osteoclasts. Later, however, the epithelioid cells functioning as osteoblasts deposited osseous substance. The grooves filled with giant cells disappeared and the cortex became smooth and thickened. The haversian canals were enlarged at earlier stages, when the congested capillaries helped to absorb the bone. At later periods, however, the reverse process was noted, inasmuch as the haversian spaces became narrowed again. With increasing formation of periosteal bone the fibrils of the periosteal connective tissue were sclerosed, and the whole periosteum was denser and thicker than in the corresponding ovariectomized guinea pigs which had not received injections of the anterior pituitary extract.

The Ribs: Here also the cartilage cells at first underwent increasing proliferation and this was again followed by retrogressive changes, which were the more marked the longer the extract was

allowed to act. The cartilaginous ground substance became loosened, swollen and disintegrated. Small cysts filled with a mucoid liquid were seen. At later stages calcification and ossification of the degenerated masses took place. The osseous parts of the ribs behaved similarly to the bony constituents of the long bones.

Discussion

During the first 2 months following ovariectomy an increased proliferation of the euhyaline cartilage of the epiphyseal disks, of the covering of the joints and of the ribs takes place. Subsequently the cartilage undergoes intensified sclerosis. Calcification and replacement of the cartilage by bone are relatively decreased. In consequence of these conditions the epiphyseal disks have become enlarged and their closure has been retarded. In the osseous spiculae of the metaphysis, the epiphysis, and in the compacta of the shaft of the long bones, resorptive predominate over appositional processes, and the bones thus become slender and thin. A slight increase of the bones in length associated with a pronounced gain in body weight is noted at the earlier stages of the experiments; at later periods, however, a gradual adjustment occurs. Permanent changes indicative of gigantism could not be observed.

Similar observations have been made by Freudenberger [4–6] and his collaborators. They found the increase in body weight and bone length of spayed rats to be relatively greater 3 months after ovariectomy than after 6 months.

The effect of ovariectomy on the cartilage may be considered as an inhibition of the ageing process of this tissue which may persist into the 2nd year of life, when ordinarily the growth potencies of the epiphyseal cartilage are completely or almost completely exhausted. This condition may be attributed to disturbances in the equilibrium of certain hormonal influences. In the first place, the lack of sex hormones resulting from ovariectomy may affect directly the course of ossification. Estrogen has been shown to cause extensive degeneration and subsequent ossification in the epiphyseal line and thus to promote the maturation of the skeleton.[7] On the other hand, ovariectomy also causes a stimulation of the anterior pituitary gland which may lead to an increased production of cartilage cells. As we have shown, this

effect is also the earliest response of the cartilage to the administration of anterior pituitary extract.[2] But the anterior pituitary hormone also promotes ossification and the question may, therefore, be raised why the stimulation of the anterior pituitary caused by gonadectomy does not suffice to restore the normal balance of proliferation and ossification of the cartilage. This may be a question of the quantitative relation between these two hormone actions; the proliferative processes resulting from the stimulation of the anterior pituitary gland in ovariectomized animals may overbalance the ossification processes in the cartilage and may make the latter more difficult. There is furthermore the possibility that species differences in the action of the anterior hypophysis, which have been shown to exist in the case of the thyroid-stimulating and gonadotropic hormones (Loeb [8-10]), may also affect the cartilage and influence the relative intensity of the growth-promoting and ossifying processes, both of which are exerted by the anterior pituitary gland. At present we have no certainty that the normal anterior pituitary hypophysis of the guinea pig will act in the same way as anterior pituitary extract of cattle. Third, there is the possibility that an increased tendency to resorptive processes in the bone is produced because of a non-specific stimulation of the thyroid gland subsequent to ovariectomy, although this effect does not seem to be very strong in the guinea pig.[11] This tendency to increased resorption of the bone might counteract the increased deposition of osseous substance in the epiphyseal disks.

The effects of ovariectomy on cartilage and bone can be modified by the administration of acid extract of anterior pituitary of cattle to the ovariectomized guinea pigs. Under these conditions the extract induces mainly retrogressive changes, namely, increased calcification and partial osseous closure of the epiphyseal disks, intensified ossification of the covering of the joints, as well as a thickening of the compacta of the shaft of the long bones and of the trabeculae of the marrow. Thus ossification predominates over proliferation. However, as compared with the action of the extract in non-ovariectomized guinea pigs, in the ovariectomized animals the processes of ossification are less accentuated. Apparently the anterior pituitary extract does not cause growth-promotion of the cartilage beyond the degree produced by ovariectomy

alone. It may be assumed that after a definite degree of growth stimulation of the cartilage has been achieved, further action of the extract causes degenerative and calcifying processes, which ultimately are followed by ossification. Thus, anterior pituitary extract of cattle may, to a certain extent, counteract the effects of ovariectomy and increase the degree of maturity in the osseous system of ovariectomized guinea pigs.

The effect of the anterior pituitary extract on bone and cartilage decreases in the course of prolonged administration in ovariectomized guinea pigs,[12] as it does in the thyroid and ovaries of non-ovariectomized, injected animals.[13–15]

Summary

During the 1st months subsequent to ovariectomy there is in immature guinea pigs an increased proliferation of the euhyaline cartilage of the epiphyseal disks and of the covering of the joints and of the ribs, whereas calcification and ossification of the cartilage are relatively retarded. At later stages the growth-promoting effect of ovariectomy on the cartilage diminishes or ceases altogether, while sclerosis of the cartilage is greatly intensified and ossification progresses, although the latter process is likewise decreased as compared with the condition in the normal animal. Gigantism does not occur, although maturation of the osseous system is delayed. If anterior pituitary extract of cattle is administered to ovariectomized guinea pigs the processes of calcification and ossification are intensified, as compared with the condition found in merely ovariectomized guinea pigs; the maturation of the skeletal tissues is therefore accelerated and the effects exerted by ovariectomy are partly counteracted.

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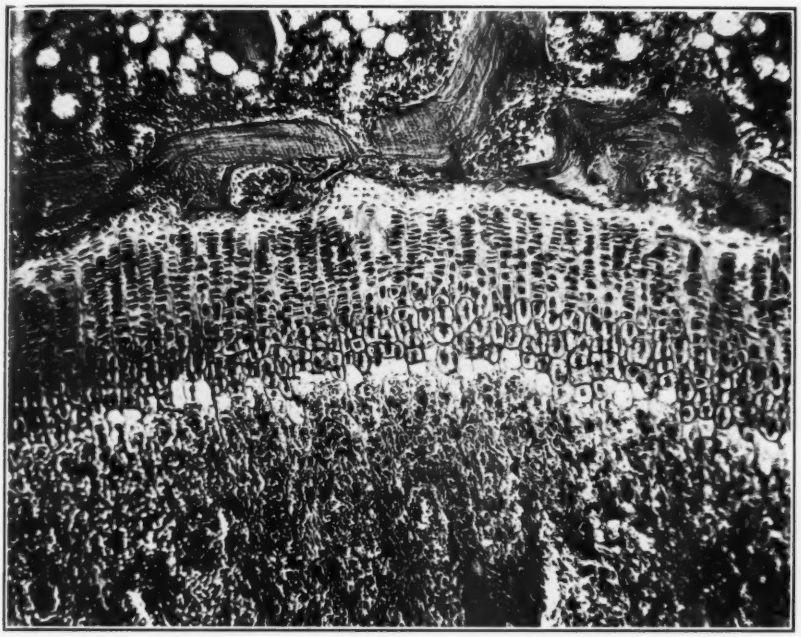
DESCRIPTION OF PLATES

PLATE 106

FIG. 1. Section through the upper part of the tibia of a guinea pig 6 weeks after ovariectomy. The weight of the animal had increased from 135 to 370 gm. during this period. Epiphyseal disk patent, no indication of osseous closure. Resting and columnar cartilage cells hyperplastic; there is only a little ground substance present. × 150.

FIG. 2. Section through the upper part of the tibia of a guinea pig 8 months after ovariectomy. The weight of the animal had increased from 140 to 750 gm. Increase in the amount of cartilaginous matrix, which is dense and fibrillar. Cell rows irregular and cells greatly reduced in number, partly shrunken and disintegrated. Ossification of the matrix and cells has progressed. × 150.



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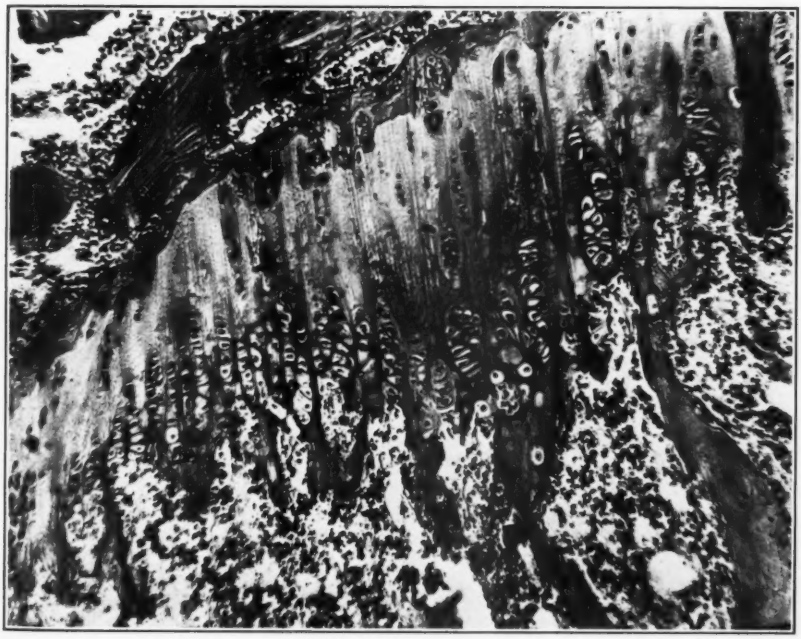
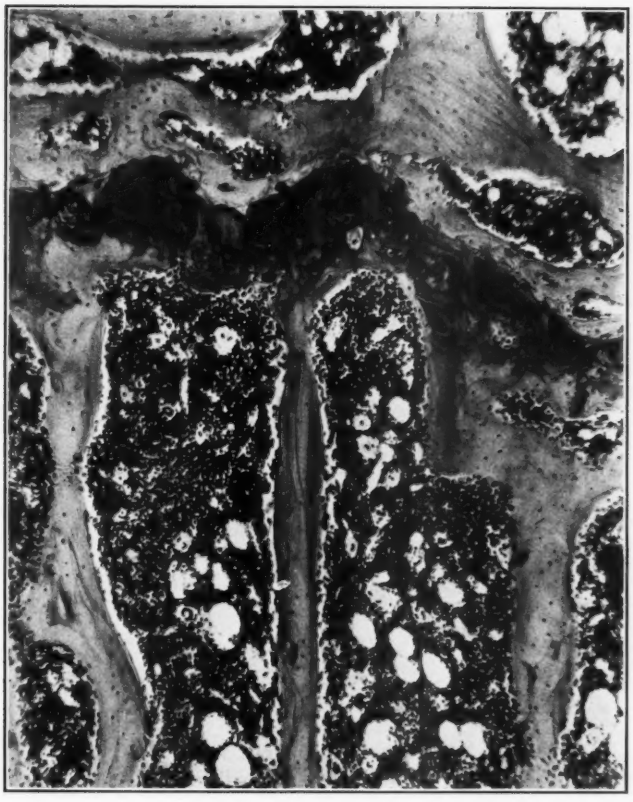


2

PLATE 107

FIG. 3. Section through the upper part of the tibia of a guinea pig 17½ months after ovariectomy. The weight of the animal had increased from 140 to 890 gm. The zone of endochondral ossification is still recognizable as a ribbon-like zone of cartilage. Rudimentary columns of cartilage cells are visible. Note the thin and elongated trabeculae in the diaphysis. × 150.



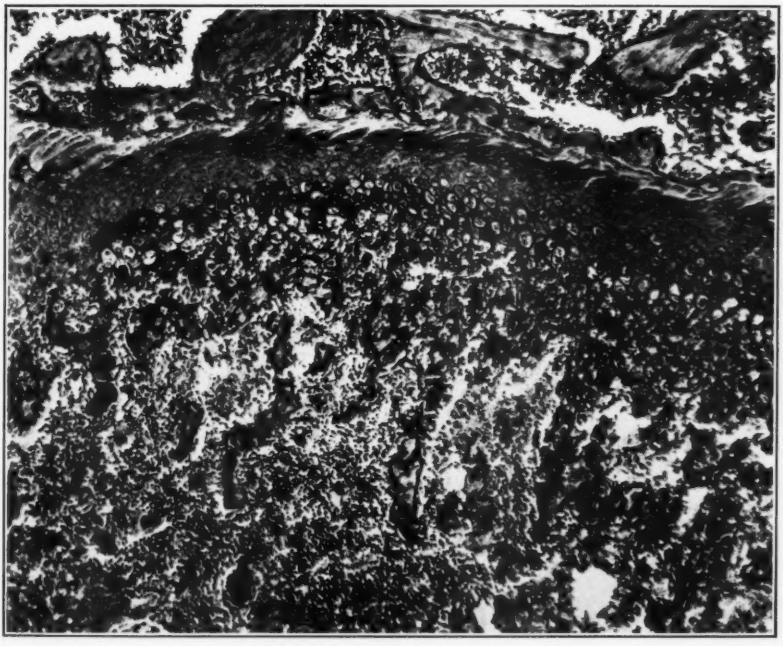
3

PLATE 108

FIG. 4. Section through the epiphyseal line of the upper tibia of a guinea pig which subsequent to ovariectomy had received 42 injections of acid extract of anterior pituitary of cattle. The weight of the animal had increased from 145 to 160 gm. during this period (compare with Fig. 1). Epiphyseal line narrower, amount of matrix slightly increased, columnar cartilage cells decreased in number, hypertrophic cartilage layer narrowed. Ossification has progressed. Osseous plug in the middle of the epiphyseal line. × 150.

FIG. 5. Section through the epiphyseal line of the upper tibia of a guinea pig which subsequent to ovariectomy had received 42 injections of acid extract of anterior pituitary of cattle. The weight of this animal had increased from 145 to 150 gm. Epiphyseal line is narrower than in Figure 4; it consists of short cartilage columns showing retrogressive changes. In the subepiphyseal layer strands of incompletely ossified cartilage are visible. × 150.

4



5

THE EFFECT OF THYROIDECTOMY AND ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT OF CATTLE ON THE GROWTH OF CARTILAGE AND BONE OF IMMATURE GUINEA PIGS *

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We have found previously that feeding of thyroid substance for periods up to 3 weeks slightly stimulates the growth of the epiphyseal cartilage and accelerates its further development into hypertrophic cartilage in immature guinea pigs.[1] On the other hand, ossification of the hypertrophic cartilage is retarded in such hyperthyroid animals, and there is also a tendency to increased resorption of bone. In order to obtain additional insight into the rôle which the thyroid hormone plays in the process of endochondral ossification and to supplement the scanty histological data available on this subject, we studied next the opposite condition, namely the influence of thyroidectomy on the growth of cartilage and bone in immature guinea pigs, and furthermore, the combined effects of thyroidectomy and of administration of acid extract of anterior pituitary extract of cattle on the osseous and cartilaginous structures, especially since in a former short communication [2] we had reported that the anterior pituitary extract may act on cartilage and bone, even in the absence of the thyroid gland.

MATERIAL AND METHODS

From 50 guinea pigs, weighing on the average 145 gm., both lobes of the thyroid gland were removed as completely as possible. Of these 50 animals, 10 were males which were used for experiments lasting only up to 3 weeks. After recuperating from the operation, 23 guinea pigs received daily intraperitoneal injections of freshly prepared extract of anterior pituitary of cattle. Two animals were sacrificed after 7, 10, 14 and 21 days respectively,

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3 after 1 month, 2 after 2, 3 after 3, and 2 after 5 months. In 5 others the injections were discontinued after 3 months, and 2 of the latter animals were allowed to survive for 1 month, 2 for 2 months, and 1 for 3 months. The remaining 27 guinea pigs did not receive any further treatment subsequent to thyroidectomy. Two of them were sacrificed after 7 and 10 days, 3 after 14, 5 after 21 days, 2 after 1 month, 3 after 2, 3 after 3, 2 after 4, 3 after 5, and 2 after 6 months. Normal guinea pigs and guinea pigs with intact thyroids which had been injected with acid extract of anterior pituitary of cattle for longer periods, and on which we have reported previously,[3] served as control material.

Observations

During the 1st weeks subsequent to thyroidectomy, a drop in weight was noted in the thyroidectomized guinea pigs, the loss being even greater in the thyroidectomized animals which had been injected with the extract.[2] For more detailed data concerning these weights after thyroidectomy, we refer to the article of Morrin and Loeb.[4]

With increasing duration of the experiments the weights of all the animals rose, after 4 months the mean weights of the normal guinea pigs being 520 gm., for the thyroidectomized animals 495 gm., and for the thyroidectomized and injected guinea pigs 485 gm. After 5 months the mean weight of the thyroidectomized guinea pigs was 10 per cent and that of the thyroidectomized and injected animals was about 5 per cent lower than that of the normal guinea pigs.

As to the gross appearances of the long bones, the tibia and the femur appeared, particularly at later stages, relatively short, owing to a flattening of the epiphysis. The length of the tibia was measured with a caliper. However, any definite differences as to its length in the thyroidectomized and normal control guinea pigs could not be established, in spite of marked histological disturbances in the former. In this connection it must be stated that a certain amount of regenerated thyroid tissue was found in all but 4 of the thyroidectomized,* and in all but 5 of the thyroidec-

* Throughout the present article we have used the term "thyroidectomized" for animals in which the thyroid gland had been completely removed, as well as for those in which some regenerated thyroid tissue was found.

tomized and injected guinea pigs. The regenerated tissue consisted of one or two small nodules of thyroid tissue which were found in the region of the operation. These nodules were as large as a small pin's head or about four times that size, but regenerates of the latter type occurred only in 2 instances. In general, the amount of regenerated thyroid tissue was larger in the guinea pigs that had received treatment with the pituitary extract. The occurrence and behavior of the thyroid regenerates have been described by Loeb and his collaborators.[4–6] However, the lack of a difference in the length of the bones in the thyroidectomized animals was not due to the thyroid remnants since no appreciable difference could be noted in the guinea pigs in which such nodules were absent.

Microscopic Examination

I. Thyroidectomized Guinea Pigs:

Epiphyseal Line: During the 1st few weeks following thyroidectomy the zone of endochondral ossification was of medium width, after 1 month it was slightly larger, and after 3 to 4 months it had again become somewhat narrower, although it was open and had an irregular line of demarcation on the side of the diaphysis. The enlargement of the epiphyseal disk went hand in hand with an increase in the number of the columnar cells; as many as 14 to 15 cells were present in one cartilage column instead of the normal number of 10 cells, whereas the number of the hypertrophic cartilage cells was normal, namely 4 in one row, or only slightly higher than normal, when 4 to 5 were found in one row. Since with the narrowing of the epiphyseal disk, which took place at later stages, the cartilage cell columns became quite irregular, an exact differential count could no longer be made.

In the cartilaginous ground substance the early changes consisted of a loosening of the fibrillar network followed by a somewhat increased deposition of calcium salts. In the 1st 3 to 4 weeks the normally basophilic chondromucoid matrix, particularly on the diaphyseal side of the epiphyseal zone, increased in quantity, became acidophilic, underwent sclerosis, and not infrequently seemed to form a plate separating sharply the zones of columnar and hypertrophic cartilage. These processes of sclerosis became more accentuated after 1 to 2 months and later. The

ground substance increased still farther and became preosseous.

During the first weeks the resting cartilage cells were slightly hyperplastic and somewhat hypertrophic. After 1 month they became more inactive, and after 3 months a normal resting condition was noted.

At the early stages the changes in the columnar cartilage cells were moderate; there was merely a slight shrinkage of the cells noticeable. From the 3rd week on, the usually gradual transition of the cells from the resting to the hypertrophic state was lacking; instead, the shrunken columnar cartilage cells were found adjoining the hypertrophic cartilage cells without the ordinary intermediary types between the two kinds of cells. At later periods the demarcation between these two layers became more marked as a result of the appearance of the above mentioned preosseous lamella in the matrix. Simultaneously, the retrogressive changes of the columnar cartilage cells had made further progress. Vacuolation and solution took place in the cytoplasm and the flattened nuclei underwent karyorrhexis and karyolysis. These changes were associated with an increase in the number of the cartilage cells (Fig. 1), some of which underwent hypertrophy and formed rounded epithelioid cells here and there. After 1 month, when both proliferation and retrogression had become intensified, irregularities in the configuration of the cartilage columns of the epiphyseal disk were noted. Later, with the increasing sclerosis of the matrix, the epithelioid cells disappeared. From the 3rd month on, and subsequently, the columnar cartilage cells were less numerous than ordinarily. A good many of them were also flat and even more atrophic than at the earlier stages. Thus, in comparison with the normal condition, the cartilage cell rows were not only shorter, but they were also evidently diminished in number.

Very soon after thyroidectomy, but more markedly after 1 month, changes in the hypertrophic cartilage cells could be observed; these cells were not quite so large as ordinarily. Their breakdown by capillaries and replacement by bone was delayed or remained incomplete, as indicated by the occurrence of unopened cartilage cells in the subepiphyseal layer. After 1 and 2 months, and still more frequently at later stages, these cartilage cells formed islands in the marrow cavity surrounded by acidophilic, epithelioid preosteocytes or enclosed in a layer of osseous

substance. Here and there a retarded direct conversion of hypertrophic cartilage into osteocytes occurred. These cartilaginous islands (Fig. 2) differ very much from the cartilaginous structures which we have frequently observed in the subepiphyseal zone under hyperthyroid conditions.[7] Whereas the latter structures are composed of large hypertrophic cartilage cells, incrusted with large amounts of calcium and arranged mainly in a lengthwise direction in the form of strands, the cartilaginous islands seen after thyroidectomy consist of distinctly smaller cartilage cells of almost mature euhyaline type, each cell being surrounded by a varying amount of cartilaginous matrix. Furthermore, these cartilaginous islands did not show a distinct orientation. Only after 4 to 6 months, in 2 out of 8 guinea pigs, a few isolated, circumscribed delicate bony plugs appeared in the epiphyseal disk as the first indication of a tendency to osseous closure. These 2 animals had weights of 560 and 590 gm. respectively. Normally, osseous closure of the epiphyseal disk of the upper tibia sets in at the age of 3 to 4 months and when a weight of 400 gm. has been reached; furthermore, this condition would have reached a still more advanced stage in normal guinea pigs that had attained an age and weight similar to those of our experimental animals.

Chondrophyte: Cells and intercellular matrix in the chondrophyte behaved similarly to the corresponding constituents in the epiphyseal line. A slight increase in the number and size of precartilage and cartilage cells was seen in cases in which proliferation in the epiphyseal disk had taken place. Incubator capsules were not observed. If retrogressive changes were present in the epiphyseal line, a few degenerated cartilage cells could likewise be detected in the lateral protuberances. At later stages, when a more normal resting condition had been reestablished, the intercellular substance was increased and dense.

Joints: In the transitional and pressure zones a slight hyperplasia and hypertrophy of the cells, as well as some retrogressive processes, were seen at the earlier periods following thyroidectomy. The zone of hypertrophic cartilage was not, or was only incompletely, being replaced by bone. Capillary loops from the bone marrow advanced between unopened cartilage cells, islands of which were seen in the epiphyseal bone marrow. As in the metaphysis, these islands were surrounded by epithelioid preosteocytes

or by a thin layer of bony substance. In the majority of animals, after 2 and 3 months, and in all animals after 4 months and later, the cells of the slightly thickened covering of the joint assumed again a resting state, and the ossification of the hypertrophic cell layer was somewhat intensified as compared with the earlier stages, but the amount of bony substance laid down was always less than under normal conditions.

Marrow Cavity: In 3 guinea pigs in which no gain or a loss of body weight was noted in the 1st weeks following thyroidectomy, the lymphoid marrow had undergone atrophy and reticular tissue had taken its place. Megakaryocytes were better preserved. No such changes were seen in animals where the body weight had increased in a regular manner. During the 1st month the trabeculae were less numerous, thinner and shorter than ordinarily, and showed only moderate calcification. Those, particularly, situated in the subepiphyseal zone were surrounded by a continuous layer of large acidophilic epithelioid cells. In contradistinction to the normal condition, when osseous substance is quickly deposited around the trabeculae, the epithelioid cells remained for a longer period connected with each other by plasmatic branches, the arrangement of these cell strands suggesting the shape of trabeculae, in which, however, the deposition of bony substance was greatly decreased. In other instances these epithelioid cells coalesced and formed multinucleated giant cells; resorptive processes took place only to a very limited extent. But after 2 months the amount of calcium and osseous substance which was laid down increased. The conversion of the epithelioid cells into osteocytes was intensified and the trabeculae became somewhat thicker. However, even as late as after 4 to 6 months the amount of bone found was less than is normal.

The Bony Shaft: In the experimental series the cortex was smooth, as compared with the normal, and showed only a few lacunar grooves, which contained single giant cells. The haversian canals were narrow. Thus, the periosteal apposition of bone predominated over the solution processes. The periosteum was poorly vascularized and, especially at the later periods, it consisted of a thick, dense, fibrillar connective tissue.

The Ribs: During the 1st weeks, and increasing in intensity with the progress of the experiment, a development of sclerosed

cartilaginous ground substance took place. Only slight to moderate retrogressive processes in the cartilage cells, such as shrinkage, hydropic swelling, vacuolation or liquefaction, were seen. Otherwise cartilage and bone behaved similarly to the corresponding tissues in the tibia.

II. Thyroidectomized Guinea Pigs which had been Injected with Anterior Pituitary Extract:

Epiphyseal Line: The zone of endochondral ossification was in all instances more regularly and more sharply demarcated toward the bone marrow, and it was narrower than in the corresponding non-injected thyroidectomized guinea pigs. The columnar cartilage cells in these animals were less numerous than they were in either the corresponding thyroidectomized non-injected or in the normal control animals. Whereas from the end of the 1st to the end of the 2nd month 14 to 15 columnar cartilage cells could be counted in a single cartilage row in thyroidectomized non-injected guinea pigs, in the thyroidectomized and injected animals the corresponding figure was 8 to 9, which is even slightly lower than the normal figure of 10. The number of hypertrophic cartilage cells likewise showed a tendency to decrease from 4 to 5 cells in the thyroidectomized animals, to 3 to 4 in the thyroidectomized and injected guinea pigs. After 2 to 3 months and later, the columnar as well as the hypertrophic cartilage cells became even more scarce, and the rows of cartilage cells became accordingly shorter.

An initial swelling of the cartilaginous ground substance was associated with a separation of its fibers. Subsequently, wider areas underwent degeneration and were impregnated with calcium salts. In the course of 2 to 4 weeks the matrix became more fibrillar, more dense and sclerosed; later it took on a preosseous character, particularly in the hypertrophic cartilage layer. In some instances this preosseous substance protruded into the zone of columnar cartilage. More calcium had been laid down in and around the cartilage cells and definite ossification of the cartilage extended from the diaphysis into the epiphysis.

The so-called resting cartilage cells showed a moderate activity at the earlier stages. They increased somewhat in number and assumed a spindle-like shape; their cytoplasm was enlarged and

sent out branched plasmatic processes. After 1 to 2 months following thyroidectomy and the beginning of the injections, these cells had increased still further in number and had also grown in size, but after 3 months the growth processes receded again, and at later periods a resting condition was reestablished, as was the case also in thyroidectomized non-injected guinea pigs.

During the 1st 4 weeks, and especially during the latter half of this period, the columnar cartilage cells underwent marked retrogressive changes, which manifested themselves in a shrinkage of the nucleus, pyknosis, karyorrhexis and karyolysis, and a vacuolation of the cytoplasm. Very large epithelioid cells were loosely and irregularly arranged in the cell rows of the cartilage and, more and more, whole cartilage cell rows were thus affected and destroyed. After 2 months the retrogressed cartilaginous areas were replaced by bone, as was indicated by the appearance of circumscribed, fairly thick osseous plugs and bridges, whereas in merely thyroidectomized guinea pigs such thick bony structures were not observed during the whole period of observation which lasted 6 months. At still later periods the localized centers of ossification became larger and more numerous (Fig. 3).

Transitional cell forms between the columnar and hypertrophic type were seen here and there, and calcium was laid down in larger quantities at the periphery of the hypertrophic cartilage cells. The comparatively small cartilage capsules, opened by congested capillary loops, which had advanced from the bone marrow, were readily replaced by bone. The ossification of the epiphyseal line was thus greatly increased as compared with the conditions in thyroidectomized non-injected guinea pigs, and it was in some instances even more accentuated than in both normal and injected animals with intact thyroids. However, notwithstanding the increased ossification, in some animals preserved islands of non-hypertrophic, non-ossified euhyaline cartilage cells, enclosed by bony material or lymphoid marrow, were seen in the subepiphyseal zone. This condition could be found even as late as 5 months after thyroidectomy in guinea pigs which had received injections of the pituitary extract. This occurrence was, however, less marked here than in the merely thyroidectomized guinea pigs.

Chondrophyte: In this series also there was a parallelism between the changes in the epiphyseal disks and those in the lateral

protuberances. At the earlier stages the matrix was loosened after 1 and 2 months; however, it appeared more collagenous and denser. At the earlier periods, and especially during the 2nd part of the 1st month, the undifferentiated perichondral connective tissue cells became not infrequently converted into precartilage and cartilage cells. Subsequently the latter enlarged or proliferated by way of amitosis. In some places four or more cells were packed together in one capsule. Such basophilic incubator capsules often degenerated. By the end of the 2nd month the stimulation of the cells gradually receded, and after 3 months a practically resting state had been reestablished. This condition did not differ at this time from the condition in normal guinea pigs or in thyroidectomized guinea pigs to which the extract had not been administered.

Joints: At the earlier periods of the experiments the cells of the transitional and pressure zones proliferated and took on a perpendicular arrangement. Numerous amitoses (Fig. 4) indicated the intensified proliferation which was associated with or followed by liquefaction or other retrogressive changes. The hypertrophic cartilage cells showed a moderate tendency to undergo calcification. Capillaries of the epiphyseal bone marrow corroded the bony border lamella, which normally separates the hypertrophic cartilage cells from the epiphyseal marrow cavity, and these could penetrate into the pressure and transitional zones of the cartilage. In some cases they grew even into the sliding zone and could reach the surface of the joint. Thus, small arthropathic lesions were produced.[8] There was no evidence of inflammatory processes. These alterations were most pronounced in the 2nd part of the 1st month, after which period they tended to recede. After 2 months an intensified calcification and ossification of the cells of the hypertrophic layer had taken place. After 3 months and more ossification was far advanced in comparison with the decreased and delayed ossification which is seen in the thyroidectomized noninjected guinea pigs.

Marrow Cavity: The connective tissue along the bony spiculae and in the subepiphyseal region proliferated and produced at first a soft, and subsequently a denser fibrous tissue. Along the osseous spiculae the mesenchymal cells could form numerous rounded epithelioid cells, which subsequently were readily con-

verted into preosteocytes and mature osteocytes. Because of this cellular apposition the trabeculae became thickened. This process progressed with further administration of the extract, so that trabeculae were finally formed which were distinctly thicker than those in the merely thyroidectomized guinea pigs.

The Bony Shaft: At earlier stages the periosteal connective tissue was loosened; later it was denser and fibrillar. The processes of absorption of bone were less accentuated than those of apposition. Thus, the cortex was fairly thick but otherwise did not show great deviation from normal.

The Ribs: Under the influence of the injections the cartilaginous ground substance became increasingly denser and sclerosed; this effect was even more marked than in the thyroidectomized non-injected guinea pigs. Retrogressive processes, affecting at first single cartilage cells and then larger areas, were distinctly intensified as compared with the merely thyroidectomized guinea pigs. At later stages calcification and ossification of such degenerated areas took place. In general, bone and cartilage of the ribs reacted in a similar way to the corresponding elements in the long bones.

In those instances in which the animals (*a*) continued to live after cessation of the injections of the anterior pituitary extract, the conditions found in cartilage and bone were similar to those noted in guinea pigs (*b*) injected without interruption during the whole period of observation, which was the same in animals (*a*) and (*b*). This would furnish additional evidence for the conclusion that the main effect of the extract is exerted during the earlier periods of the injections, and that the potency of the extract decreases with increasing administration, a fact which has been established in the thyroid and ovaries by Loeb and Friedman,[9] and in the cartilage and bone by us.[3]

Discussion

In thyroidectomized young guinea pigs proliferation of the cartilage takes place at the same rate as in normal guinea pigs, or the growth is somewhat increased as compared with the normal condition, at least at an early period following thyroidectomy. However, the conversion of columnar into hypertrophic cartilage cells is disturbed, transitional forms between these two kinds of cells developing only incompletely; moreover, the hypertrophic

cartilage cells are smaller than ordinarily, and their replacement by bone is definitely delayed. The cartilaginous ground substance increases gradually in amount and assumes, progressively, an acidophilic and preosseous character. On the other hand, the processes leading to osseous closure of the epiphyseal disk are retarded. Deposition of bone around the trabeculae is decreased.

A comparison of the effects of thyroidectomy and of ovariectomy on cartilage and bone shows that although both interferences cause an increased proliferation of cartilage and a delayed replacement of cartilage by bone, they greatly differ in several respects. The chondromucoid character of the cartilaginous ground substance seen in normal guinea pigs is maintained in ovariectomized guinea pigs longer than under normal conditions, whereas the cartilaginous matrix becomes prematurely preosseous under the influence of thyroidectomy. After ovariectomy the proliferation of the cartilage is greater than after thyroidectomy; the gradual but steady transformation of columnar into hypertrophic cartilage remains unchanged, and the full maturation of the latter is only delayed but is not affected in kind in the spayed animals, whereas these processes are actually disturbed in the thyroidectomized guinea pigs, as is evidenced by the lack of a gradual and complete differentiation of the cartilage. The replacement of the hypertrophic cartilage by bone, although retarded and diminished in amount in ovariectomized animals, takes place at an evenly balanced and regular rate, in contradistinction to conditions following thyroidectomy, where the breakdown of the cartilage cells and their subsequent ossification are incomplete and irregular. Finally, in ovariectomy, resorption of osseous substance predominates over the apposition of bone and causes the formation of thin and slender osseous structures; on the other hand, after the removal of the thyroid gland the resorption of bone is diminished, as is also the apposition of osseous tissue. The bones of thyroidectomized animals appear, therefore, thicker than those of ovariectomized guinea pigs.

Administration of anterior pituitary extract of cattle to thyroidectomized guinea pigs causes increased retrogressive changes in the epiphyseal cartilage, followed by a more marked ossification than is found in merely thyroidectomized or in normal guinea pigs; proliferation may be stimulated in the cartilage of the joints.

The calcification of the hypertrophic cartilage cells and their subsequent replacement by bone are greatly increased over that observed in thyroidectomized guinea pigs which had not been injected with pituitary extract. The formation of bony substance in the covering of the joints and around the osseous trabeculae of the marrow cavity is likewise intensified. In the joints the increasing ossification leads, at later periods, to a repair of the arthropathic lesions which were initiated at earlier periods. Thus, anterior pituitary extract of cattle causes a growth stimulation of the cartilage as well as an increased ossification, both in thyroidectomized and in ovariectomized guinea pigs. Under both circumstances a combination effect takes place. However, following thyroidectomy the proliferation of the cartilage is less pronounced after the administration of anterior pituitary extract than it is following ovariectomy. On the other hand, retrogressive changes and subsequent ossification are more marked after injection of pituitary extract in thyroidectomized guinea pigs than in spayed animals.

Thus, in spite of the continued lengthwise growth of the long bones, thyroidectomy causes changes in those tissues in which this growth takes place. Such changes consist in (1) accelerated sclerosis of the cartilaginous matrix; (2) failure of the epiphyseal cartilage to undergo regular and full hypertrophy; and (3) incomplete and delayed ossification of the abnormal cartilage.

These findings suggest a number of problems which have been under discussion since von Eiselsberg [10] demonstrated the retardation of growth of thyroidectomized goats and sheep. These problems can be divided into four groups: (1) the specificity of the growth effect of thyroidectomy and correspondingly also of the thyroid hormone; (2) the primary changes in bone and cartilage following thyroidectomy; (3) the significance of the age factor in determining the effects of thyroidectomy; and (4) the possible effect of thyroid deficiency on the general endocrine balance and the effect which a disturbance in the latter might in turn exert on the growth of bones.

Concerning the first problem, Hofmeister [11] held the effect of thyroidectomy on cartilage to be a specific one, but the more recent investigations of Hammett [12, 13] in rats, Todd and his collaborators [14] in sheep, and Dye and Maughan [15] in dogs, all based

on gross examinations, favor the assumption of a non-specific metabolic influence of thyroidectomy. Thyroidectomy reduces the metabolism and is, therefore, supposed to decrease the amount of nutritive material available to the cells for the maintenance of normal growth. From this viewpoint it would then be necessary to prove that the histological changes observed after thyroidectomy can be correlated with nutritional deficiencies. The increased sclerosis of the ground substance and the atrophy of the columnar cartilage cells seen after thyroidectomy could well be due to an adverse nutritional condition. This assumption seems to be supported also by the observation that columnar cartilage cells and cartilaginous matrix may, in certain respects, behave similarly in underfed guinea pigs. However, in certain other respects the changes noted in undernourished and thyroidectomized guinea pigs differ. We shall report on these findings at a later date.*

Concerning the second problem, Dieterle,[16] on the basis of a histological study of the bones of a child which had suffered from congenital athyreosis and of a single experiment in a thyroidectomized kitten, pointed out that the primary disturbance caused by thyroidectomy consists in the inability of the bone marrow to bring about a breakdown of the cartilage which would lead to secondary changes in the latter. These conclusions, which were confirmed by Hagenbach [17] in more extensive experiments in cats, were based on the arbitrary contention that the same principle underlies both periosteal and endochondral bone formation and on the observation that periosteal bone formation was decreased simultaneously with endochondral ossification. However, Landauer,[18] Dye and Maughan,[15] Binswanger,[19] and Todd and his collaborators,[14] have demonstrated that the periosteal bone formation is only slightly affected by thyroidectomy. On the other hand, Hofmeister [11] noted in thyroidectomized rabbits a primary specific degenerative process in the epiphyseal cartilage, "chondrodystrophia thyreopriva," which caused secondarily a delayed ossification. In thyroidectomized tadpoles Terry [20] observed increased growth of the cartilaginous vertebrae and calcification of the

* Silberberg, Martin, and Silberberg, Ruth. Changes in cartilage and bone of immature female guinea pigs due to undernourishment and the processes of repair following a period of refeeding. *Arch. Path.*, 1940, **29** (in press).

cartilage, but only a trace of ossification of the cartilage, even as late as 7 months after the period of life when metamorphosis would have taken place in normal tadpoles. More recently, Coryn [21, 22] stressed the significance of the absence of hypertrophic cartilage cells in an athyreotic child, a condition which he tried to reproduce in rabbits. One thyroidectomized and 1 normal rabbit were given thyroid hormone. His finding that administration of this hormone causes an increased hypertrophy of the cartilage agrees with the results of our investigations.[1] However, by injecting thyroid hormone into thyroidless animals, he did not reproduce a condition of true thyroid deficiency, and, correspondingly, no definite conclusions can be drawn from this experiment as to the effect of thyroidectomy. On the other hand, our experiments make it possible to conclude that thyroidectomy affects directly the growth of the cartilage and that the process of ossification of the epiphyseal line is only secondarily involved. This, however, does not preclude the possibility that bone formation may be likewise directly affected, owing to the metabolic disturbance. But this decrease in bone formation may be obscured by the simultaneous decrease in resorptive processes following thyroidectomy. The result of both of these occurrences would be a fairly normal appearance of the trabeculae and the cortex which we have actually observed at later stages.

As to the significance of the age factor in the effectiveness of thyroidectomy, this has been established, but only on the basis of gross examinations. Conditions seem, however, to be different in different species. In dogs Binswanger [19] found thyroidectomy to be more effective if performed at an early age, whereas it does not affect growth if performed after the animal has reached the age of 4 months. Hammett,[23] on the other hand, found the growth retardation in rats to be greater if thyroidectomy was performed at the age of 100 days rather than at the age of 23 days.

Since our present material consists of guinea pigs which have all been thyroidectomized at about the same age, we are not in a position to state whether the age factor plays any determining rôle in this species. In view of the fact, however, that in spite of the marked histological changes which could be observed in the bones of our animals the lengthwise growth of the tibia was not markedly affected, it is quite possible that we did not choose the

most favorable age for the initiation of a measurable growth retardation. Furthermore, there is at least the possibility that as in sheep (Todd and collaborators [14]) and in tadpoles (Allen [24]), so also in guinea pigs the effect of thyroidectomy does not become apparent before a certain time has elapsed following the operation, and that our experiments may not have been extended long enough. It is conceivable that the retardation of growth might be demonstrable in bones in which osseous union of epiphyses and diaphyses has not yet occurred and in which proliferation of cartilage still takes place, while the growth-inhibiting effect of thyroidectomy would not be manifest in bones in which growth had come to a standstill (Zuck [25]). In guinea pigs such a retardation of growth under the influence of thyroidectomy could take place only at later stages, because, as stated above, at early stages thyroidectomy has the opposite effect on the epiphyseal cartilage — it intensifies its growth.

It is also conceivable that the presence of a small amount of regenerated thyroid tissue in the majority of instances may make possible the lack of inhibition of bone growth. In our experiments we would have to deal with a hypothyreotic condition rather than with a complete lack of thyroid function. Such a possible influence of a small remnant of thyroid tissue must be considered, especially in view of the fact that the long bones possess a greater resistance toward growth retardation, caused by the lack of thyroid hormone, than other tissues (Hammett [26, 27]). However, in the animals in which thyroidectomy had been complete, the bones did not behave differently from those in which a remnant was found. It seems, therefore, very improbable that the lack of growth inhibition was due to the incomplete operation.

We have finally to estimate the interference of other endocrine glands in the effect of thyroidectomy on bone growth. As to the thymus, no definite statement can be made. There are, however, certain indications that the anterior pituitary may be stimulated as a result of thyroidectomy and that this condition may lead to secondary changes. The increase in the number of cartilage cells at the end of the 1st and during the 2nd month following thyroidectomy may be due to this secondary condition. The stimulation exerted by the anterior pituitary must be quite strong at this time, because it is able to overcome the lowering of the metabolism

caused by lack of thyroid hormone. But, after all, the latter condition must in the end limit the proliferation. On the other hand, injections of anterior pituitary extract in thyroidectomized guinea pigs did not lead to marked proliferation of the cartilage; on the contrary, widespread degeneration occurred and the ossifying influence of the extract became manifest in the narrowing of the epiphyseal zones and in the formation of numerous osseous plugs.

According to Flower and Evans,[28] the interaction of the effects of thyroidectomy and of injections of anterior pituitary hormone creates a condition in which the growth-inhibiting effects of the former are compensated for by the action of the latter. This conclusion, which appears in accordance with data based merely on gross findings, led Flower and Evans to assume that growth retardation subsequent to thyroidectomy is caused by hypopituitarism resulting from thyroid deficiency. The microscopic study, however, does not show an annulment of the characteristic effect of thyroidectomy by simultaneous administration of anterior pituitary hormone. There are, rather, the effects of both factors recognizable. In our guinea pigs, where the growth-promoting effect of the acid extract on the cartilage was less accentuated than the retrogressive changes, the extract not only did not counteract the effect of thyroidectomy, but it worked in the same direction as thyroid deficiency, which acts at least partly by creating in the tissues a state of malnutrition, thus increasing the tendency of the cells to undergo retrogression. Since retrogressive changes in the cartilage are always favorable to ossification, under the additional stimulus of the extract the degree of ossification produced may surpass that of normal animals, as well as of animals with intact thyroids which had been treated with the anterior pituitary extract. There is another reason why anterior pituitary hormone should not counteract the effect of thyroid deficiency. Loeb,[29] Siebert and Thurston,[30] and Siebert and Smith [31] have shown in guinea pigs, and Schwartzbach and Uhlenhuth [32] in amphibia, that anterior pituitary hormone is not able to raise the metabolism in thyroidectomized guinea pigs. Consequently, growth changes in the cartilage, as far as they are due to lowered metabolism resulting from thyroidectomy, cannot be repaired by the administration of anterior pituitary hormone. If, then, anterior

pituitary extract is able to maintain growth after thyroidectomy, this does not occur by counteracting the effect of thyroidectomy, but its action must be due to a different mechanism, based on the growth-promoting action of the extract.

There is, then, a fundamental difference between the effects of the anterior pituitary extract in thyroidectomized guinea pigs on the one hand, and in ovariectomized guinea pigs on the other. Thus, in ovariectomized guinea pigs the pituitary extract is able to accelerate maturation of the cartilage and thus to counteract the effect of ovariectomy, an effect which is not noticeable in thyroidectomized animals.

Summary

Thyroidectomy in immature guinea pigs exerts a direct effect on the growth zones. It causes an accelerated sclerosis of the cartilaginous ground substance and inhibits the complete differentiation of columnar cartilage cells into hypertrophic cartilage cells. Proliferation of the cartilage cells is maintained and may even be temporarily increased. Endochondral ossification is secondarily affected because of the inadequate supply of mature cartilage cells available for ossification. At later stages the amount of osseous substance in the shaft of the long bones shows little deviation from the normal. This may be due to a decreased resorption balancing a possible decrease in apposition of osseous material, rather than to a complete lack of influence of thyroid deficiency on periosteal and trabecular bone formation. The changes in cartilage and bone may be partly due to the lowered metabolism existing in hypothyreotic conditions; however, the temporary increase in the proliferation of the epiphyseal cartilage may be a consequence of the stimulation of the anterior pituitary gland subsequent to thyroidectomy.

Anterior pituitary extract of cattle exerts its influence on cartilage and bone in guinea pigs in which the thyroid gland has been completely or almost completely removed. Under these circumstances increased growth and retrogressive changes in the cartilage, followed by an enhanced ossification, take place. However, the differentiation of the cartilage remains incomplete after thyroidectomy, even if anterior pituitary hormone is administered, and normal conditions are not restored. There takes place, there-

fore, under these conditions, a summation of the effects of thyroidectomy and anterior pituitary hormone.

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DESCRIPTION OF PLATES

PLATE 109

FIG. 1. Section through the upper tibia of a female guinea pig which had been sacrificed 3 weeks subsequent to thyroidectomy. No regenerated thyroid tissue was found at autopsy. The weight of the animal had increased from 165 to 225 gm. The epiphyseal line is somewhat enlarged, the cartilaginous ground substance augmented. Irregular arrangement of cartilage cell rows which are partly lacking in some areas is present. Atrophy of columnar cartilage cells and rather abrupt transition of cartilage cells of columnar type into those of hypertrophic type is also seen. × 150.

FIG. 2. Section through the upper tibia of a female guinea pig which had been killed 3 months following thyroidectomy. Some regenerated thyroid tissue was present at autopsy. The weight of the guinea pig had increased from 135 to 445 gm. The epiphyseal line is irregularly configurated and the replacement of cartilage by bone is irregular. Dense cartilaginous matrix is present. In the subepiphyseal zone there is an islet of euhyaline cartilage surrounded by a layer of bony material. × 150.

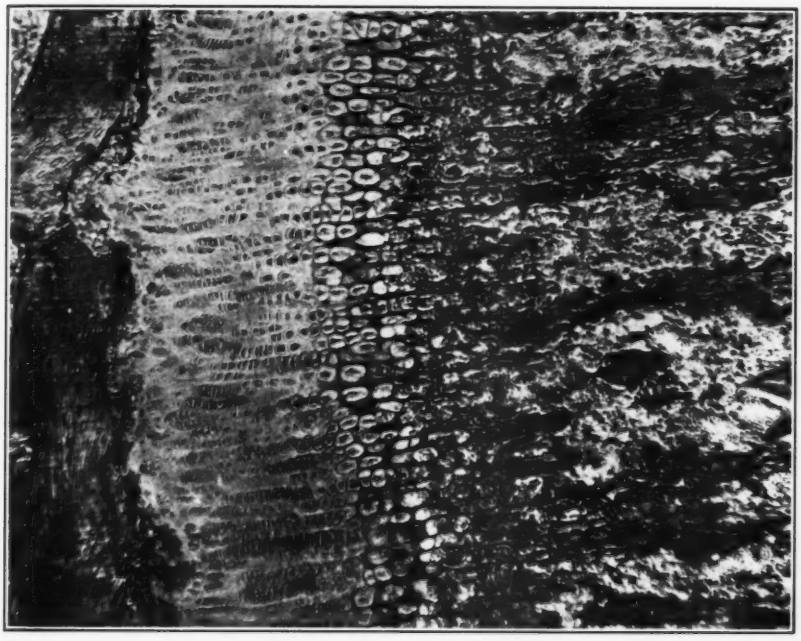

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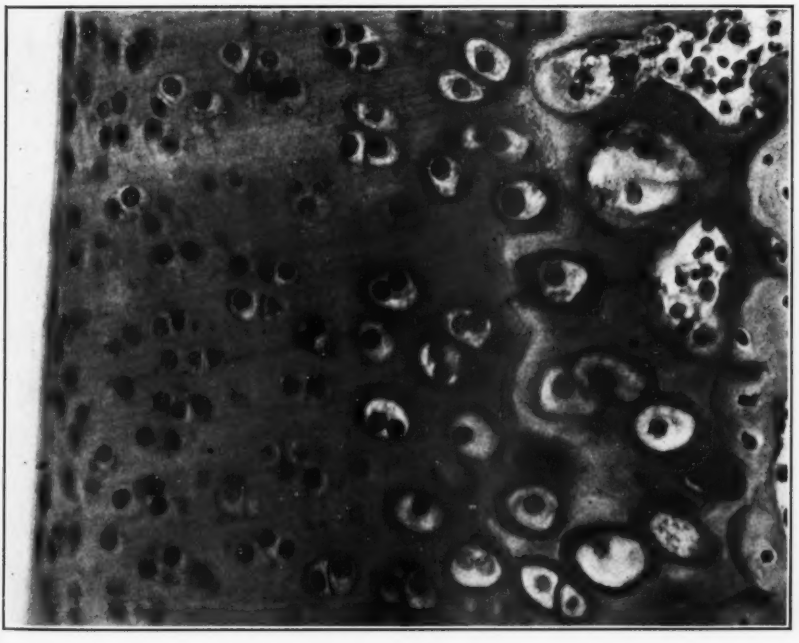
PLATE 110

FIG. 3. Section through the upper tibia of a female guinea pig which had been thyroidectomized and subsequently had received 90 injections of anterior pituitary extract of cattle on 90 days, 1 cc. being given daily. The weight of the guinea pig had increased from 140 to 465 gm. No regenerated thyroid tissue was found at autopsy. The epiphyseal line is fairly regular and the cartilaginous ground substance is increased and sclerosed. Note the thick osseous plugs and bony bridges traversing the cartilaginous disk. × 150.

FIG. 4. Section through the knee joint of a female guinea pig which subsequent to thyroidectomy had been injected daily for 3 weeks with 1 cc. of anterior pituitary extract of cattle, when the animal was killed. The weight of the guinea pig had increased from 155 to 160 gm. At autopsy some regenerated thyroid tissue was seen. The cells of the uppermost, the sliding zone, are flat, while there is noticeable proliferation and hypertrophy of the cells in the transitional and pressure zones, where numerous amitoses can be observed. The preosseous matrix reaches into the hypertrophic cell layer. Capillary loops corrode the bony border lamella. × 225.

3



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